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Bosshardt, Stephen Carl, Ph.D.

The Louisiana State University and Agricultural and Mechanical Col., 1990

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BRUGIA PAHANGI: EFFECTS OF MATERNAL FILARIASIS ON THE RESPONSES OF THEIR PROGENY TO HOMOLOGOUS INFECTION

A Dissertation

Submitted to the Graduate Faculty of the
Louisiana State University and
Agricultural and Mechanical College
in partial fulfillment of the
requirements for the degree of
Doctor of Philosophy

in

Interdepartmental program in
Veterinary Medical Sciences

by
Stephen C. Bosshardt
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December, 1990

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ABSTRACT

The effects of *in utero* and neonatal exposure to maternal *Brugia pahangi* infections on the development of homologous infections, immune responses, and pathologic lesions was studied in age-matched progeny of infected and uninfected female jirds. High IgG antibody titers to *B. pahangi* antigens were present in the sera of progeny from infected mothers. These neonatal titers to homologous antigens were shown to be of maternal origin, and did not alter the cellular responses of uninfected progeny as measured by *in vitro* antigen-stimulated blastogenesis and *in vivo* pulmonary granulomatous inflammatory responses to antigen-coated beads. Studies were conducted to measure age-related differences in susceptibility, lymphatic lesion formation, and antibody responses to *B. pahangi* infection in 2-, 6-, 10-, and 15-week old jirds from uninfected mothers. Significant reductions in quantities of testicular and intralymphatic worms recovered and antibody responses to soluble antigens in jirds infected at 2 weeks of age had no measurable effect on susceptibility or lymphatic lesion severity expressed as the ratio of intralymphatic thrombi formed per intralymphatic worm. Challenge infections in 2- or 4-week old progeny from *B. pahangi*-infected and uninfected female jirds yielded equivalent adult worm recoveries and microfilaremias. Lymphatic lesion severity expressed as tne ratio of intralymphatic thrombi formed per

intralymphatic worm recovered was similar in all groups. Offspring infected at either 2 or 4 weeks of age from infected mothers exhibited significantly lower serum IgG antibody titers to B. pahangi antigens compared to infected progeny of uninfected mothers at 5-8 weeks postinfection. Infected offspring from infected mothers displayed significantly fewer antigen-specific splenic plaque-forming cells at 5 weeks postinfection than infected control progeny. Qualitative and quantitative reductions in serum antibody reactivity to B. pahangi antigens were also demonstrated by Western immunoblot at 8 weeks postinfection in infected progeny of infected females. These results suggest that a partial immune tolerance to B. pahangi antigens develops in infected offspring of infected female jirds. These modulated antibody responses had no measurable effect on the establishment of adult worms, microfilaremias, lymphatic lesion development, or antigen-specific pulmonary granulomatous inflammatory responses.

INTRODUCTION

Lymphatic Filariasis - Distribution and Life Cycle

Human lymphatic filariasis is a disease caused mainly by the filarial nematodes Wuchereria bancrofti, Brugia malayi, and Brugia timori. It has been estimated that 90 million people are currently infected with one of these three species of lymphatic filarial parasites (World Health Organization, 1989). The predominate species is W. bancrofti, which is endemic in many tropical and subtropical areas of Latin America, Africa, and Asia. B. malayi is found mostly in Southeast Asia, while foci of B. timori infection have only been described from the islands of Timor, Flores, Roti, and Alor of Indonesia (Kurihara and Oemijati,1975; World Health Organization, 1989). One other filarial species, B. pahangi, must also be considered a potential human pathogen based on both successful experimental transmission to humans (Edeson et al., 1960) and reports of microfilariae with B. pahangi-like acid phosphatase activity from human subjects (Palmieri et al., 1985). Unfortunately, infective larvae and subsequently adult parasites were not produced from these B. pahangi-like microfilariae which would have made possible the confirmation of human infections with this parasite. B. pahangi was originally described as a species of lymphatic filarial parasite in carnivores within the geographical area

around the Pahang River, Malaysia (Buckley and Edeson, 1956). Its zoonotic potential is still unknown.

All filariae causing human lymphatic filariasis exhibit complex life histories. Their typical nematode life cycles involve five sequential developmental stages (L_1-L_5) within an invertebrate intermediate host and a vertebrate definitive host (Noble and Noble, 1976). The following is a generalized description of a filarial nematode life cycle based on experimental studies of various species of filariae with special emphasis, where possible, on the genus Brugia.

Sexual reproduction by mature adult male and female filariae (L_5) occurs within the lymphatic vessels and lymph nodes of the vertebrate definitive host (Ewert, 1971; Elbihari and Ewert, 1971; Suswillo et al., 1982). Fertilized eggs mature in utero within the female worm to a sheathed L_1, or microfilaria (Taylor, 1960a). Microfilariae are the infective stage for the hematophagous arthropod intermediate host once they have been released by the adult female worm and enter the bloodstream.

A variety of mosquito species, including those in the genera Anopheles, Aedes, Culex, and Mansonia, can serve as intermediate hosts (vectors) for either Wuchereria or Brugia (reviewed by Wharton, 1963; and Denham and McGreevy, 1977). Ingested microfilariae exsheath, penetrate gut and enter the arthropod hemocele where they migrate to the thoracic musculature to further develop (Taylor, 1960b; Schacher,

1962a). Two molts occur (L_1-L_3) within 8-17 days depending on both the species of mosquito and filarid (Taylor, 1960b; Schacher, 1962a). The resultant L_3 is infective for the vertebrate definitive host, and available for natural transmission upon migration back to the head and mouthparts of the vector.

Infective L_3 are not injected into the bloodstream during vector feeding, but must exit the mosquito labium and gain entry through the puncture wound left behind by the mosquito fascicle (Ewert, 1967; McGreevy et al., 1974). Larvae rapidly migrate to the lymphatic vasculature (Suswillo et al., 1982), and a majority reside within either the regional lymph nodes or afferent/efferent lymph vessels of these nodes (Ewert, 1971; Elbihari and Ewert, 1971; Suswillo et al., 1982). Studies on experimental B. pahangi infections suggest that the final molt to the immature adult stage (L_5) requires approximately 24 days in jirds (Ash and Riley, 1970) and 35 days in cats (Schacher, 1962b). Sexual reproduction occurs soon after the final molt and microfilariae are produced. The period of time between initial experimental infection with B. pahangi and the ability to detect microfilariae in the blood, known as the prepatent period, has been reported as 53 days in cats (Denham et al., 1972a), 56 or 57 days in jirds (Ash and Riley, 1970; Ash, 1973), 75 days in Macaca mulatta (Denham, 1974), and 84 days in humans (Edeson et al., 1960). One

report of experimental infections of human volunteers with B. malayi noted a prepatent period of 17 weeks in the only patent infection observed (Dondero et al., 1972). Wuchereria bancrofti has an estimated prepatent period in humans of 7 months (Partono, 1987).

Pathogenesis of lymphatic filariasis

As a clinical disease, lymphatic filariasis is expressed in a wide variety of symptoms and manifestations (Otteson, 1980; 1984; Partono, 1987). Categories of clinical manifestations observed in filariasis patients have been established (Otteson, 1980), but the symptoms of some patients still defy this categorization. The exact mechanisms which lead to one form or another of filarial disease are unclear, although immunologic reactivity of the host to filariae is thought to play an important role (Otteson, 1980; 1984; 1989). The following discussion will concentrate on filariasis as a clinical entity. The varied immunological reactions of infected humans and experimental animals will be addressed more completely in subsequent sections.

Of the 5 categories of clinical manifestations of filariasis established by Otteson (1980), the most common symptom is that of lymphatic inflammation (lymphadenitis and lymphangitis) associated with "filarial fevers". These symptoms are typical signs of acute filariasis due to

infection with either B. malayi (Turner, 1959; Edeson et al., 1960; Dondero et al., 1972) or W. bancrofti (Wartman, 1947). The main difference in disease caused by these two species of filarial parasites is the affected site within infected individuals (Partono, 1987). Filariasis due to W. bancrofti infections can involve both the extremities and genitalia (Wartman, 1947), while B. malayi mainly involves the distal aspects of affected legs and arms (Turner, 1959). The inflammation and associated local lymphstasis and edema occurring with these episodes of "filarial fever" usually resolve with time. Symptoms have been known to last, however, for as many as 15-16 years after infected individuals have left an area endemic for filariasis (Trent, 1963).

Experimental studies into the pathologic changes in lymphatic vessel structure of infected hosts suggests the participation of both immune and non-immune mediated inflammatory events. The most commonly observed lymphatic change associated with acute filarial infection is lymph vessel dilation (Ah and Thompson, 1973; Rogers and Denham, 1974; Rogers et al., 1975; Vincent et al., 1980a; Crandall et al., 1982; Snowden et al., 1986). This lymphangiectasis is often accompanied by regional lymph node enlargement (Ah and Thompson, 1973; Crandall et al., 1982; Snowden et al., 1986) caused by hyperplasia of both T and B cell components of the immune system (Rogers et al., 1975; Vincent et al.,

1984). Endothelial hyperplasia and thickening, perilymphangitis and fibrosis occur along with cellular infiltrates consisting mainly of neutrophils, eosinophils (Ah and Thompson, 1973; Rogers and Denham, 1974), macrophages, lymphocytes, and occasionally plasma cells (Vincent et al., 1980a; 1984). Intralymphatic granulomatous thrombi are also commonly present around dead worms and microfilariae, and sometimes progress to calcified lesions (Ah and Thompson, 1973; Vincent et al., 1980a). All of these pathological changes are are the result of adult worms within the lymphatic vasculature, and can produce localized edema in some experimental models (Crandall et al., 1982; 1987; Snowden et al., 1986). Studies of the lymphatic pathology of B. pahangi infections in nude mice have indicated that most of these acute inflammatory events can be found independent of T cell-mediated immune responses (Vincent et al., 1984). Additional studies in cats (Rogers and Denham, 1974; Denham et al., 1983), ferrets (Crandall et al., 1987; 1990), dogs (Snowden et al., 1986; Snowden and Hammerberg, 1987), and jirds (Klei et al., 1981; 1982; 1987) have shown that existing infection or immunization can produce either an anamnestic-type inflammatory response to inoculation of L_3, or a hyporesponsive state of granulomatous reactivity suggested to be due to immunosuppression in chronic infections (Klei et al., 1981; 1987). The administration of immunosuppressive agents or

inflammatory substances during B. pahangi infections in cats has also led to diminution or exacerbation of lymphatic dilation, respectively (Dean et al., 1983). Filarial antigen-specific and non-specific inflammatory events can occur during primary infection.

Recurrent episodes of acute lymphadenitis, lymphangitis, and edema, with decreasing resolution of lesions, presumably leads to the chronic stage of lymphatic obstructive disease known as elephantiasis. Chronic bancroftian filariasis can lead to an irreversible enlargement of the entire affected extremity or genitalia, hydrocele, orchitis, and/or chyluria. Lesions due to chronic B. malayi infections most commonly affect only the distal portions of the infected appendages (Partono, 1987). Elephantiasis patients usually do not have microfilariae in their blood.

Two other classifications of clinical disease include individuals who are typically amicrofilaremic. The first group of infected individuals display an asthma-like condition, often occurring nocturnally, referred to as tropical pulmonary eosinophilia (Lie, 1962; Otteson et al., 1979; Otteson, 1980; 1984). These individuals also display an allergic sensitization to filarial antigens derived from the microfilarial stage (Otteson et al., 1979). Blood eosinophil (Van der Sar and Hartz, 1945; Wong and Guest, 1969) and serum IgE antibody levels to microfilarial

antigens are elevated (Otteson et al., 1979). The microfilariae produced, however, are killed before a measurable amount of them can reach the blood. These killed microfilariae are found in lesions containing a high percentage of eosinophils known as Meyers-Kouwenaar bodies that may be pathognomonic for occult filariasis (Lie, 1962). The second group of amicrofilaremics, the "endemic normals", constitute perhaps the most enigmatic proportion of a population indigenous to areas endemic for filariasis. Though exposed to filarial infection, they fail to show any clinical or parasitological signs of filarial parasitism. This "endemic normal" group purportedly displays protective immunity against filarial infections (Otteson, 1984; Philipp et al., 1988), although post-patent occult filariasis cannot be excluded (Otteson, 1984).

The last of the clinical categories of Otteson (1980) are asymptomatic like the "endemic normal" group, but the presence of circulating microfilariae belies their infected status. This group is usually a large proportion of the population within areas endemic for filariasis (Otteson, 1980). The exact mechanisms behind this group's hyporesponsive clinical state to filarial infection is unclear, although reduced immune responses to filarial antigens is a common finding (Otteson, 1980; 1984; Piessens et al., 1987).

Immune Responses to Filarial Parasites

Immune responses to filarial nematodes can be most logically approached within the context of the categories of clinical manifestations of Otteson (1980). The discussion of immune responses of infected persons will, where appropriate, include reports from experimental filarial infections in animal models. Experimental studies in animal models provide a majority of the evidence for the induction of protective immunity in permissive hosts. Vaccination against filariae in permissive or natural hosts will be approached separately at the end of this discussion.

The cell-mediated immune responsiveness of individuals living within areas endemic for lymphatic filariasis has most often been measured by *in vitro* lymphocyte proliferation assays to mitogens and/or filarial antigens from one or more life cycle stages. Human peripheral blood mononuclear cell blastogenic responses to either heterologous adult antigens (Otteson et al., 1977) or homologous microfilarial antigens (Piessens et al., 1980a) were, in general, significantly reduced in microfilaremic versus amicrofilaremic filariasis patients infected with either *W. bancrofti* or *B. malayi*, respectively. Lammie et al (1988), however, found that 50% of microfilaremic *W. bancrofti*-infected Haitians showed significant lymphocyte blastogenic responses to *B. pahangi* antigens with stimulation indices of > 2. Evidence from all of these

reports indicated that nonresponsive lymphocyte blast transformation in these patients was parasite antigen-specific, and had no effect on responses to other antigens such as tuberculin purified protein derivative, streptokinase-streptodornase (Otteson et al., 1977; Piessens et al., 1980a), or the mitogen phytohemagglutinin (Lammie et al., 1988).

One study in W. bancrofti-infected humans did observe reduced blastogenic responses of peripheral blood mononuclear cells to concanavalin A in acute and chronic filariasis patients without microfilaremias when compared to uninfected controls (Narayanan et al., 1986). Narayanan et al (1986) further showed that amicrofilaremic patients exhibiting hydrocele as their only clinical symptom demonstrated suppressed blastogenic responses to phytohemagglutinin and pokeweed mitogen, as well as concanavalin A, when compared to peripheral blood lymphocytes from uninfected controls. Experimental studies with B. pahangi have also demonstrated reductions in lymphocyte blastogenic responses to mitogens as a consequence of chronic infections in jirds. Splenocytes from jirds harboring patent B. pahangi infections do not proliferate as well as those from uninfected jirds in response to the T cell mitogens concanavalin A and phytohemagglutinin (Portaro et al., 1976; Lammie and Katz, 1983a; 1983b), or to the B cell mitogen E. coli

lipopolysaccharide (Lammie and Katz, 1983a). Lymph node cells from infected jirds, however, showed reduced blastogenic responses only to the B cell stimulant pokeweed mitogen when compared to uninfected jirds (Lammie and Katz, 1983a). This depression of splenocyte blastogenic responsiveness in infected jirds has been linked to either the generation of spleen cells reactive with adult worm antigens in blast assays (Portaro et al., 1976) or decreases in both splenic plaque-forming cell responses to sheep red blood cells and blast transformation responses to parasite antigens occurring near the onset of microfilaremia (Lammie and Katz, 1983b). The presence of cells capable of suppressing splenocyte lymphoproliferative reactivity to mitogens in infected jirds has been demonstrated by the alleviation of this suppressed state following the depletion of cells adherent to nylon wool or plastic (Portaro et al., 1976; Lammie and Katz, 1984), or by carbon particle-magnet techniques (Portaro et al., 1976). In vivo treatment with cyclophosphamide was also successful in reducing the observed suppression of splenocytes from microfilaremic jirds to mitogens, but not antigens (Katz and Lammie, 1984). These data suggest that the spleen cell capable of suppressing mitogenic reactivity of splenocytes in microfilaremic jirds is of the monocyte/macrophage lineage.

Antigen-specific splenocyte blastogenic responses of jirds infected with B. pahangi become reduced, in comparison

to uninfected jirds, around the time of patency (Lammie and Katz, 1983b). This suppressed state of splenic blastogenesis to parasite antigens in infected jirds was not observed in lymph node cells, and did not coincide with any alteration in _in vivo_ delayed-type hypersensitivity responses to dinitrofluorobenzene as measured by increases in ear thickness (Lammie and Katz, 1983b). Removal of plastic-adherent cells did not reconstitute normal _in vitro_ spleen cell blastogenic responses to parasite antigens in chronically infected jirds (Lammie and Katz, 1984a). Depletion of nylon wool adherent splenocytes, or splenocytes bearing receptors for histamine, peanut agglutinin, soybean agglutinin, or jird IgG Fc did, however, enhance splenic blastogenesis to parasite antigens in infected animals (Lammie and Katz, 1984a). These data suggest the presence of an antigen-specific suppressor T cell in the spleen, but not the lymph nodes, of B. _pahangi_-infected jirds expressing microfilaremia. Interestingly, microfilaremic filariasis patients have also demonstrated antigen-specific suppressor T lymphocytes (Piessens et al., 1982). Adherent suppressor cells and suppressive serum factors which can depress _in vitro_ blastogenic reactivity to homologous microfilarial antigens when compared to amicrofilaremic patients have also been demonstrated (Piessens et al., 1980c).

Filariasis patients with patent W. _bancrofti_ infections have also displayed other deficiencies in cell-mediated

immune responses besides reduced lymphocyte proliferation to parasite antigens or mitogens. Peripheral blood mononuclear cells from microfilaremic individuals are deficient in their ability to produce both interleukin 2 and gamma interferon compared to amicrofilaremic patients in an antigen-driven *in vitro* system (Nutman et al., 1987). Production of these lymphokines was similar in both groups of patient's cells in response to phytohemagglutinin or tuberculin purified protein derivative. Removal of either $OKT8^+$ T cells or plastic-adherent cells did not enhance the elaboration of either lymphokine in this assay (Nutman et al., 1987). Spleen cells from microfilaremic *B. pahangi*-infected jirds likewise produced lower amounts of interleukin 2-like growth factor than either autologous lymph node cells or spleen cells from *B. pahangi*-immunized or infected, prepatent jirds (Leiva and Lammie, 1989a). Removal of spleen cells bearing histamine receptors from these suppressed splenocyte populations from microfilaremic jirds restored their ability to produce interleukin 2 to *B. pahangi* antigen stimulation. This restoration of interleukin 2 production by spleen cells of patently infected jirds, however, may not be sufficient to overcome their suppressed blastogenic responses to parasite antigens, as exogenous interleukin 2 added to the splenocyte cultures in this study had no effect on antigen-driven lymphoproliferation (Leiva and Lammie, 1989a).

Chronic human filarial infection eventually leads to irreversible lymphatic vessel damage due to repeated local traumatic inflammatory responses. These chronic granulomatous responses have been characterized as containing epithelioid and multinucleated giant cells, plasma cells, and eosinophils (Meyers et al., 1976). The exact nidus for these reactions remains elusive, although live or dead parasites and/or their products are the most likely candidates (Otteson, 1980; Meyers et al., 1976). Male jirds inoculated subcutaneously with B. pahangi L_3 develop lymphatic-dwelling adult parasites which can be associated with intralymphatic granulomatous lesions remarkably similar in cellular composition to those observed in humans (Vincent et al., 1980; Jeffers et al., 1987). The formation of these intralymphatic lesions in B. pahangi-infected male jirds can be either enhanced by homologous immunization prior to infection (Klei et al., 1982) or diminished by pre-existing intraperitoneal infections (Klei et al., 1981; 1987). In addition, reduced numbers of these granulomatous lymph "thrombi" are found in jirds infected for 140-150 days (Klei et al., 1988; 1990). This apparent down-regulation of lymph thrombi formation observed in chronically-infected jirds is hypothesized to occur in a fashion similar to the modulation of egg granuloma size in Schistosoma mansoni-infected mice harboring chronic infections (Boros et al., 1975). The cell type responsible

for the regulation of filariae-induced thrombus formation, if it is indeed controlled by immunoregulatory mechanisms similar to the delayed-type hypersensitivity reactions within the schistosome egg granuloma, could be a T suppressor cell (Doughty and Phillips, 1982). It is interesting to note that the redcued lesion severity measured in B. pahangi-infected jirds (Klei et al., 1990) coincides with the reported appearance of antigen-specific, histamine receptor-bearing suppressor cells in the spleen during the onset of microfilaremia (Lammie and Katz, 1983b). These reports strongly suggest that the antigen-specific modulation of granulomatous hypersensitivity to B. pahangi in jirds is due to T cells. Direct experimental evidence for the existence of immunoregulatory networks controlling intralymphatic thrombi formation in filariae-infected hosts, however, is not available.

Cellular cytotoxicity reactions, mediated through antibody-dependent mechanisms, are another host immune response to filarial parasites. Several in vitro studies with sera from amicrofilaremic subjects have indicated that microfilariae of both W. bancrofti and B. malayi can be killed by antibody-dependent cellular cytotoxicity reactions involving homologous IgG antibodies and neutrophils (Mehta et al., 1981; Aiyar et al., 1982). Interestingly, eosinophils demonstrated less adherence to microfilariae than neutrophils (Aiyar et al., 1982), and were apparently

ineffectual at killing microfilariae once they had adhered (Mehta et al., 1981). The specificity of this microfilaricidal response of neutrophils *in vitro* resided in the IgG fraction of sera from amicrofilaremic patients, as neutrophils from endemic normals and even microfilaremic individuals were equally effective in killing microfilariae (Mehta et al., 1981). Furthermore, sera from microfilaremic patients did not promote appreciable leukocyte adherence to microfilariae (Subrahmanyam et al., 1978; Mehta et al., 1981; Aiyar et al., 1982). Adherence of leukocytes to microfilariae can also be mediated by complement components (Aiyar et al., 1982; Piessens and daSilva, 1982), although Aiyar et al (1982) failed to consider that the observed loss of adherence after treatment of sera at 56° C for thirty minutes might be due to complement inactivation.

Cellular cytotoxicity reactions against infective L_3 also occur through antibody-dependent mechanisms. Immune serum from patients with elephantiasis, tropical pulmonary eosinophilia, symptomatic amicrofilaremic filariasis, and uninfected endemic individuals promoted *in vitro* buffy coat cell adherence to L_3 of *B. malayi* (Sim et al., 1982). These buffy coat cells damaged L_3 *in vitro* (Sim, 1981) to the extent that they are not infective for jirds (Sim et al., 1982). Rat neutrophils and macrophages adhere and are cytotoxic for *B. pahangi* L_3 *in vitro* as measured by trypan blue exclusion in the presence of fresh normal rat serum or

a Protein A-purified IgG fraction of immune serum (Chandreshekar et al., 1985). Eosinophils adhere to infective larvae of W. bancrofti in vitro in the presence of serum from microfilaremic or amicrofilaremic patients (Higashi and Chowdhury, 1970). Higashi and Chowdhury (1970) were unable, however, to measure any effects of adherent eosinophils on larval motility in vitro. Adherence of human leukocytes to L_3 appears to mediated primarily by IgG and/or IgM antilarval antibodies (Higashi and Chowdhury, 1970; Sim et al., 1982), while rat leukocyte adherence can be mediated by IgG antibodies to filariae and/or complement components (Chandreshekar et al., 1985).

Humans infected with filarial parasites, in general, display elevated levels of serum IgM, IgE, and to a lesser extent IgG (Sim et al., 1983). A relatively clear distinction exists, as was observed in blastogenic responsiveness, between serum antibody levels to filarial antigens in microfilaremic and amicrofilaremic filarial infections in humans (reviewed by Otteson, 1984). A strong inverse correlation has been demonstrated between the presence of serum IgM and IgG antibodies to microfilariae and the presence of circulating microfilariae (Wong and Guest, 1969; McGreevy et al., 1980; Piessens et al., 1980b). Antimicrofilarial antibodies, especially of the IgG isotype, are well known to participate in the initial adherence step of in vitro antibody-dependent cellular cytotoxicity

reactions against microfilariae (Subrahmanyam et al., 1978; Mehta et al., 1981; Aiyar et al., 1982).

Serum IgG antibodies produced in response to filarial infections show a number of interesting characteristics. Naturally occurring serum IgG antibodies have been demonstrated in filariasis patients which can inhibit IgE-mediated histamine release from basophils in an antigen-specific manner (Otteson et al., 1981a). Such regulation of immediate hypersensitivity responses may be lacking, or deficient, in individuals experiencing tropical pulmonary eosinophilia, although no experimental proof of this exists. Recognition of filarial parasite antigens by subclasses of serum IgG antibodies from asymptomatic microfilaremic individuals differs from that of amicrofilaremic patients exhibiting chronic symptoms of lymphatic obstructive filarial disease. Asymptomatic patients have demonstrated large quantities of serum IgG_4, and antibodies within this subclass of IgG reacted with a broad range of filarial parasite antigens. Symptomatic filariasis patients, on the other hand, showed relatively reduced serum IgG_4 levels and antigen recognition profiles in Western immunblots developed specifically for IgG_4 antibodies (Hussain et al., 1987). Additionally, serum antibodies of both IgG_1 and IgG_3 subclasses reacted with filarial antigens either above or below antigens of approximately 68kd molecular weight in symptomatic versus asymptomatic patients, respectively

(Hussain et al., 1987). These data suggest that IgG subclass antibody interactions with filarial antigens may reflect either the immune and/or clinical status of the host. Finally, IgG and IgE antibodies are known to overlap in their specificity for filarial antigens in Western immunoblots (Hussain and Otteson, 1985), but the serum of only 3 out of 8 putatively immune "endemic normals" had IgE antibodies reactive with a 43kd molecular weight antigen recognized by IgG antibodies in 100% of these apparently immune individuals (Freedman et al., 1989). These latter data strengthen the contention that IgG antibodies are the major isotype participating in antifilarial immune effector networks.

Serum IgE antibodies to the microfilarial sheath could not be demonstrated in one study of filariasis patients (McGreevy et al., 1980). The presence of antimicrofilarial IgE antibodies is implied, however, by the high levels of IgE antibodies measured by basophil-histamine release assays to both metabolic and somatic microfilarial antigens, especially in patients displaying tropical pulmonary eosinophilia (Otteson et al., 1979). The relative levels of antifilarial IgE:IgG antibodies recognizing identical parasite antigens in Western immunoblots was found to be the highest among tropical pulmonary eosinophilia patients (Hussain and Otteson, 1985). Serum IgE antibodies to filarial antigens have also proven to be more species-

specific than IgG antibodies for the immunodiagnosis of human helminthic diseases caused by nematode parasites (Weiss et al., 1982). IgE antibodies are known to play an important role in *in vitro* antibody-dependent cellular cytotoxicity reactions to schistosomula (reviewed by Butterworth, 1984), but the role of IgE in protective immune responses to the L_3 or microfilariae of filarial parasites remains unclear.

Filariae and/or their antigens are also known to directly affect the immune responses of their hosts. Spleen cells from uninfected jirds displayed reduced mitogen-stimulated blastogenesis when co-cultured with splenocytes from jirds harboring chronic *B. pahangi* infections (Lammie and Katz, 1984b). This non-specific suppression of splenocytes from uninfected jirds by those from infected jirds was greater when suppressing splenocytes were first activated *in vitro* by incubation with either mitogen or *B. pahangi* antigens (Lammie and Katz, 1984b). Further studies have indicated that *B. pahangi* microfilarial antigens of > 150kd molecular weight can inhibit lymph node cell proliferative responses to both concanavalin A and adult *B. pahangi* antigens (Leiva and Lammie, 1989b). Immunosuppression caused by high molecular weight antigens of various *B. malayi* life cycle stages have also been observed in homologously infected humans (Piessens et al., 1987). Circulating antigens of both *B. malayi* (Wenger et

al., 1988; Weil, 1988) and B. pahangi (Weil et al., 1990) have also been found which contain phosphorylcholine. Phosphorylcholine alone, and B. malayi antigens containing phosphorylcholine, have demonstrated a dose-dependent ability to suppress in vitro mitogen-induced lymphoproliferation of peripheral blood mononuclear cells from normal and infected individuals via a putative T cell mediated effect (Lal et al., 1990). Conversely, microfilarial extracts of B. malayi have been shown to have mitogenic activity for the $T4^{+}$ subset of human T cells (Wadee and Piessens, 1986). Lymphocyte activation has also been demonstrated with various fast protein liquid chromatography fractions of B. malayi adult worm antigens, including a fraction containing phosphorylcholine (Lal et al., 1987). The potential role of other demonstrable circulating filarial antigens (Dasgupta et al., 1984; Maizels et al., 1985; Huijan et al., 1987; Weil et al., 1987; Weil, 1988) and immune complexes (Karavodin and Ash, 1980; Lunde et al., 1988; Prasad and Harinath, 1988) in regulating host immune responsiveness is not known.

Evidence for the expression of protective immune responses to filarial parasites by humans is, at best, inferential. A proportion of populations living within areas endemic for lymphatic filariasis remains uninfected by all clinical and parasitological measures, despite exposure to filariae-carrying vectors. These people must, therefore,

be segregated into the category of putatively immune individuals known as "endemic normals" (Otteson, 1980; 1984; Philipp et al., 1988). Attempts to detect genetic loci within the A or B regions of the HLA associated with susceptibility to filarial infection were unsuccessful, although familial clustering of filariasis was observed (Otteson et al., 1981b). Another study, however, did show a genetic association between HLA-A and -B haplotypes and the expression of elephantiasis (Chan et al., 1984). Recent evidence has emerged concerning IgG antibody recognition of adult, microfilarial, and L_3 antigens suggesting that protective immune responses in endemic normal individuals may be directed against a 43kd molecular weight L_3 antigen (Freedman et al., 1989). It is difficult to imagine, however, that protective immunity against a parasite as complex as the filariae could arise through antibody recognition of one parasite antigen. Indeed, the variability associated with measurement of acquired protective immune responses in humans, such as previous infections with filariae or other helminths, makes conclusions from data difficult to make (Nutman, 1989).

Experiences gained through the study of experimental permissive hosts, such as the cat or jird, have indicated that induction of protective immunity to filariae is a highly variable and complex event. Evidence of naturally-acquired immunity to B. pahangi infections in cats has been

observed only after repeated inoculations (Denham et al., 1972b; 1983). Conversely, B. pahangi-infected jirds became either more susceptible to establishment of challenge infection (Klei et al., 1980), or showed no changes in immunity to repeated infections (Klei et al., 1981; 1987; 1988).

Successful vaccination of permissive or natural hosts against filariae has been accomplished using infections terminated by drug therapy. Dogs have been vaccinated against Dirofilaria immitis by means of chemically-abbreviated infections (Grieve et al., 1988). Multiple inoculations of D. immitis L_3 terminated with ivermectin treatment approximately 60 days later reduced the establishment of adult heartworms from challenge infections by almost 99% in this study. Dogs immunized by this regimen showed dramatically increased serum antibodies to homologous L_3 compared to unimmunized dogs (Grieve et al., 1988). Previous vaccination of ferrets against D. immitis using a similar protocol yielded approximately 85% reduction in the establishment of adult parasites (Blair and Campbell, 1981). Jirds given prophylactic treatment with flubendazole, and then 5 inoculations with B. pahangi L_3 over a 63 day period, displayed less than 50% protection against challenge infection compared to untreated challenge controls (Chusattayanond and Denham, 1984).

Induction of protective host immunity has also been studied using radiation attenuated larval vaccines. Cats vaccinated with 10 krad irradiated B. pahangi L_3 exhibited an 80% reduction of challenge infection adult worm burdens compared to unvaccinated cats, although this level of immunity was achieved only after a very cumbersome immunization protocol (Oothuman et al., 1979). Levels of homologous protection as high as 76% and 91% were observed in jirds immunized with irradiated L_3 of B. pahangi (Chusattayanond and Denham, 1986) and B. malayi (Yates and Higashi, 1985), respectively. Rhesus monkeys vaccinated with 20 krad irradiated B. malayi L_3 failed to exhibit patent infections following challenge infection (Wong et al., 1969). The difficulties in detecting occult infections and quantitation of adult worm burdens in this large animal model, however, does not allow the separation of protective immunity to challenge infection from antimicrofilarial immunity (Wong et al., 1969). Interestingly, jirds immunized with irradiated Litomosoides carinii L_3 demonstrated 98% and 71% reduced adult worm burdens compared to untreated jirds when challenged with L. carinii or B. pahangi, respectively (Storey and Al-Mukhtar, 1982). This latter result insinuates that cross-protective immunization against multiple species of filarial parasites using an irradiated larval vaccine composed of just one filarial species is possible.

Other experiments on the induction of protective immune responses against filariae have used either microfilariae or their extracted antigens as immunizing agents. Extracts of B. malayi microfilariae have been used successfully in jirds as an immunogen to promote both partial protective immunity against development of adult parasites (approximately 55% reduction compared to controls), as well as enhancing clearance of circulating microfilariae in infections that developed to maturity (Kazura et al., 1986). Two other studies using microfilarial injections as a method of immunization deserve special attention due to their implications in vaccine development for human use. In ferrets, microfilarial immunization with B. malayi produced consistent partial protective immunity against adult worm establishment, but also increased the rate at which lymphatic pathologic changes occurred following homologous infections (Crandall et al., 1990). Immunizations of jirds with either B. pahangi microfilariae, or homologous adult worm antigens in adjuvant, also led to increased lymphatic lesion severity compared to unimmunized controls with no reduction in adult worm burdens following homologous challenge infections (Klei et al., 1982). It should be noted that the previously discussed acquired protective immunity in cats after multiple B. pahangi inoculations (Denham et al., 1983) resulted in a transient lymphedema of the inoculated leg immune cats. These results present an

obvious caveat concerning the pathological consequences of inducing protective immune responses in humans, especially if the engendered immunity is not absolutely sterile.

Mice are not permissive hosts for the full development of Brugia spp. infections initiated by subcutaneous inoculation of infective L_3 (Laing, 1961; Ahmed, 1967). They do, however, support development of the L_3 of Brugia malayi to the L_4 stage. The L_3 stage is thought to be the major target of protective immune responses and stage-specific immune responses against the L_3 can be studied in the nonpermissive murine host. Furthermore, immunity in mice is hypothesized to represent the putative immunity exhibited by the "endemic normal" portion of a population which exhibits no clinical or parasitological signs of filarial parasitism.

Mice can be readily immunized against the larval development of B. malayi by a variety of methods including live L_3, L_4, or adult B. malayi (Carlow and Phillip, 1987), microfilariae (Carlow and Phillip, 1987; Hayashi et al., 1989), soluble extracts of L_3 (Carlow and Phillip, 1987) or microfilariae (Hayashi et al., 1989), and radiation-attenuated L_3 (Abraham et al., 1989; Hayashi et al., 1989). The expression of immunity against developing larvae of B. malayi challenge infections in mice has been measured by more rapid killing of developing larvae (Carlow and Phillip, 1987) and both decreases in larval challenge infection

survival and increased retardation of growth of L_3 within diffusion chambers implanted into immunized mice (Abraham et al., 1989). Furthermore, immunity engendered in mice can be adoptively transferred to naive mice in nylon wool nonadherent T cell populations (Hammerberg et al., 1989; Hayashi et al., 1989).

That the nature of protective immunity to filarial parasites is T cell dependent, at least in experimental hosts, has been proven by studies in the nude mouse. In general, adult mice are refractory to B. pahangi infections initiated by subcutaneous inoculation of L_3 as mentioned above (Laing, 1961; Ahmed, 1967). The nude mouse, however, is completely susceptible to L_3 challenge infection, and allows full parasite development to the adult stage (Vincent et al., 1980b; 1982). These facts alone suggest a dependency on intact T cell immunity for the elaboration of protective responses against B. pahangi. Confirmation of these findings was obtained through reconstitution of T cells in homozygous nude mice by syngeneic thymus grafts prior to infection (Vickery et al., 1983). Graft recipients allowed no B. pahangi adult worms to develop from the L_3 challenge infection; and infusion of infection-primed splenocytes from refractory heterozygous nude donors into homozygous nude recipients yielded similar results. Transfer of immune serum with high antibody titers to both adult and larval antigens, however, failed to transfer

protection against challenge infection to nude mice as measured by adult worm recoveries (Vickery, 1983). The expression of protective immune responses would, therefore, seem to depend on the activation of T cells reactive with parasite antigens present on developing forms of the parasite. The exact antigens required for such protective immunity may not be so easily found.

The murine model may be a very useful tool for the identification of protective antigens of filariae. A recent study using murine monoclonal antibodies has identified excretory/secretory antigens of B. malayi ranging from 26-28 kd molecular weight that can induce protective immunity to larval challenge infection in the absence of antibody production against the immunizing antigens (Hammerberg et al., 1989). Conversely, mice immunized with irradiated L_3 of B. malayi produced antibodies to both internal and external antigens of both L_3 and L_4 stages, while challenge-infected control mice produced serum antibodies reactive with only internal structures of these larval stages (Abraham et al., 1989). Participation of serum antibodies in protective immune responses is questionable due to a reduced ability of serum transfer to engender protective immunity compared to adoptive cell transfer (Vickery et al., 1983; Hayashi et al., 1989). The murine model of antifilarial immunity is relatively new to this field, but the wealth of knowledge concerning the murine immune system

and the availability of commercial reagents for murine studies increases the potential for benefits derived from the use of this model.

Fetal and Neonatal Immune Responsiveness

The immune system begins its development early in fetal life. The fetal and neonatal immune system is immature compared to that of an adult, especially in the efferent or effector arm (Goldman et al., 1985; Wilson, 1986). This immaturity of the fetal and neonatal immune system is counteracted through a variety of passive maternal contributions (Ockleford and Dearden, 1984; Powell et al., 1984). The relative level of pre- versus postnatal maternal immunological assistance varies between mammalian species, but the unified goal is to protect the progeny from infectious agents without compromising the subsequent immune responsiveness of the neonate.

Lymphoid cells develop from a common pluripotent stem cell thought to arise first from the yolk sac, and later from the fetal liver (Moore and Owen, 1967; Owen and Ritter, 1969). The fate of these lymphocyte progenitors as T or B cells is dependent on their migration to, or residence within, specific fetal organs such as the liver or thymus (Asma et al., 1977; Gathings et al., 1977; Rosenthal et al., 1983).

The human fetal thymus initially develops by approximately 6 weeks of age (Wilson, 1985), and is

populated by developing thymocytes by 9 weeks of age (Asma et al., 1977). T lymphocytes bearing the sheep erythrocyte receptor can be demonstrated in the thymus at 9-11 weeks postconception (Asma et al., 1977; Stites and Pavia; 1979). Lymphocyte blastogenic responsiveness to phytohemagglutinin can first be detected in the fetal thymus at 10 weeks of age, the spleen at 12 weeks, and in the blood by approximately 15 weeks (Stites et al., 1974). Another hallmark of T cell reactivity, responsiveness to alloantigens in mixed lymphocyte reactions, can be detected in thymic cells by 13 weeks of age (Stites et al., 1974). Fetal spleen and thymus cells respond to in vitro concanavalin A stimulation both by proliferating and interleukin 2 production by 17-21 weeks of age (Bodeker et al., 1982). Production of interleukin 2, but not the expression of interleukin 2 receptors, is thought to be deficient at this time because the addition of exogenous interleukin 2 greatly enhances fetal proliferative responses to concanavalin A in vitro (Bodeker et al., 1982). Numbers of T cells (Deibel et al., 1983), and in vitro proliferative responses to phytohemagglutinin and alloantigens, are slightly higher in cordblood mononuclear cells than in adult peripheral blood cells (Bryson et al., 1980). Neonatal T cell-mediated responses are, however, deficient in many respects compared to those of adults (Stiehm et al., 1979). The elaboration of gamma interferon by newborn human

mononuclear cells, as induced by *in vitro* stimulation with phytohemagglutinin, was negligible compared to adult levels (Bryson et al., 1980). Production of other types of interferon in response to viral antigens, however, was found to be equivalent in newborn, cordblood, and adult mononuclear cells (Bryson et al., 1980). Human neonatal cell-mediated lympholysis has also been characterized as being approximately half that of adult values (Granberg et al., 1979; Granberg and Hirvonen, 1980). Recent studies have indicated that reduced neonatal lymphocytolytic activity compared to adult cells is not due to decreased production of lymphotoxin, although production of tumor necrosis factor was significantly lower in neonatal cells (English et al., 1988).

Studies of cord blood cells have also shown a higher T_4:T_8 lymphocyte ratio than in adult peripheral blood (Yachie et al., 1981; Jacoby and Oldstone, 1983), with adult ratios of helper to suppressor T cells being attained by 3-5 years of age in humans (Yachie et al., 1981). Furthermore, $OKT4^+$ and $OKT8^+$ subsets in cordblood and newborn human mononuclear cells have been defined with different functions than those normally ascribed to these same subsets in adult cells. The helper subset ($OKT4^+$) of $OKT3^+$ T lymphocytes from human cord blood lacks a measurable ability to regulate B cell differentiation in an *in vitro* pokeweed mitogen-driven system (Yachie et al., 1981). The $OKT4^+$ T lymphocyte

subset from cord blood or newborn blood has, conversely, suppressed the differentiation of B cells using this assay (Yachie et al., 1981; Jacoby and Oldstone, 1983). Subsequent investigations, however, discovered a radiation-resistant subpopulation of the $OKT4^+$ subset of human newborn cells that did display B cell helper activity in addition to an $OKT4^+$ radiation-sensitive suppressor cell population (Jacoby and Oldstone, 1983). Various other reports contend that neonatal $OKT8^+$ suppressor cells are the major reason for reduced helper activity (Yachie, et al., 1981) or outright suppression of adult B cell differentiation and immunoglobulin synthesis in pokeweed mitogen-stimulated cultures (Rodriguez et al., 1981).

Pre-B cells containing only cytoplasmic IgM heavy chain can be demonstrated in the human fetal liver by approximately 8 weeks of gestation (Gathings et al., 1977). Surface immunoglobulin positive B cells, but not plasma cells, are prevalent during the intrauterine fetal development that is normally devoid of antigenic stimulation (Hayward and Lydyard, 1979). Immature B cells expressing surface IgM receptors are detectable by about 10 weeks of fetal age (Gathings et al., 1977). Surface IgD immunoglobulin is concomitantly expressed on the surface of IgM positive "mature" fetal B cells by about 12 weeks of age (Lawton and Cooper, 1979). Surface IgG- or IgA-bearing B lymphocytes can also be demonstrated at 12 weeks of

gestation indicating that isotype switching has occurred (Gathings et al., 1977; Lawton and Cooper, 1979). Fetal B cells can also be found which display three or more isotypes of immunoglobulins, unlike the single isotype expression of adult IgG^+ or IgA^+ B cells (Gathings et al., 1977). Fetal pre-B cells can be detected in the bone marrow, the site of adult lymphocyte hematopoiesis, by 14-15 weeks of age (Gathings et al., 1977; Rosenthal et al., 1983). Subsequent B cell maturation into antibody-secreting plasma cells is dependent on antigenic stimulation of individual clones.

Neonatal phagocytic cells have also been shown to have functional deficiencies in their ability to fight infectious diseases (Wilson, 1986). Relative numbers of monocytes (Berman and Johnson, 1978) and rosette-forming neutrophils (Masuda et al., 1989) in human cord blood have been shown to be higher and lower, respectively, than adult peripheral blood values. Neonatal monocytes have proven an ability equal to that of adult monocytes in Fc and complement receptor-mediated phagocytosis, lysozyme secretion, and myeloperoxidase production, although activity of the neonatal lysozyme is diminished by adult standards (Berman and Johnson, 1978). Studies in mice have further proven that thymic Ia^+ accessory cells are fully capable of stimulating T cell proliferation through the process of antigen presentation just after birth (Lu et al., 1980). Neonatal neutrophils, on the other hand, have a decreased

chemotactic capability, and adherence through Fc receptor-mediated mechanisms is less than that of adult neutrophils (Masuda et al., 1989). Neonatal neutrophils have, however, shown in vitro priming by recombinant human granulocyte-macrophage colony-stimulating factor that is equal to that of adult neutrophils as measured by oxidative burst metabolism and chemotaxis (Cairo et al., 1989). Neonatal neutrohils, therefore, seem capable of performing their respective functions, but lack the ability of adult cells to coordinate their attack to antigenic or infectious stimuli. Neonatal monocytes also appear to perform their antigen presentation functions, but apparently do so to an immature lymphoid system as described above.

Maternal contributions to the immature responsiveness of the fetal and neonatal immune system consist mainly of the passive transfer of immunoglobulins. The relative importance of pre- versus postnatal immunoglobulin transfer varies between mammalian species (Hogarth, 1982). In animals where in utero immunoglobulin transfer predominates, passive transfer of maternal immunoglobulins to the circulation of the fetus has been shown to be a receptor-mediated event involving the passage of primarily IgG either across the yolk sac (Hogarth, 1982) or placenta (Jollie, 1985). Postnatal transfer of maternal immunoglobulin across the neonatal rat intestinal epithelium has also been shown to be receptor-mediated (Jakoi et al., 1985). The majority

of human maternal immunoglobulin is received transplacentally (Powell et al., 1984). The potential exists for the transfer of maternal cells or cell-mediated immune responses to the fetus (Field and Caspary, 1971; Thong et al., 1974;), but confirmation of this phenomenon is difficult. Prenatal acquisition of cell-mediated neonatal immune responsiveness to microbial agents from the mother has been documented in only a few isolated cases (Russell, 1975; Horton et al., 1976). Documentation of actual cell transfer across the placenta has indicated, however, that very few maternal cells traverse the unaltered placenta in mice (Hunziker et al., 1984).

Postnatal acquisition of passive maternal immunity in humans is comprised of both specific immunoglobulins consisting mainly of IgA (Ogra and Ogra, 1978a; Goldman et al., 1982), non-specific factors such as lactoferrin and lysozyme (Goldman et al., 1982), and phagocytic leukocytes (Ogra and Ogra, 1978b; Goldman et al., 1982). Immunoglobulins and other factors acquired after birth generally provide only local protection against viral and bacterial agents in the gut and respiratory tract (Hogarth, 1982; Powell et al., 1984). Maternal cell transfer across the human gut epithelium, or transfer of cell-mediated responses through cytokines like T cell transfer factor, is difficult to confirm (Powell et al., 1984).

The developing immune system of the fetus and neonate undergoes a variety of maturational stages. These stages are very orderly, and are aided by maternal contributions which supplement the generally immature neonatal immune responses. During this entire period, the establishment of immunologic tolerance to "self" antigens is shaped (Nossal, 1983; Roser, 1989). The process of determining "self" from "non-self" is a very important and complex cascade of events beyond the scope of this review. More important than the induction of tolerance to "self" antigens is the fact that active maternal immune responses to infectious organisms during gestation can lead to fetal and/or neonatal immune system tolerance to "non-self" antigens.

Several experimental studies in rodents using erythrocyte antigens as an immunogen have shown that maternal antigenic stimulation during pregnancy can lead to a diminution in the primary splenic plaque-forming cell responses of their progeny to the homologous antigens when compared to the progeny of unstimulated mothers (Auerbach and Clark, 1975; Yamaguchi et al., 1983; Izuchi et al., 1985; Sharmonov et al., 1986; Koshimo et al., 1989). Various mechanisms have been proposed to account for this observed maternal suppression of primary plaque-forming cell responses including the transfer of specific antibodies (Izuchi et al., 1985; Sharmonov et al., 1986) or immune complexes (Auerbach and Clark, 1975) via the milk,

transplacental transfer of antigen (Yamaguchi et al., 1983), or the induction of identifiable suppressor T cells (Koshimo et al., 1989). A study using ovalbumin-immunized female rats has also indicated that specific maternal antibodies transmitted in the milk resulted in reduced primary antibody responses as measured by serum levels of antiovalbumin IgG, IgM, and IgE antibodies (Okamoto et al., 1989). These reduced serum antiovalbumin antibody levels occurred only in progeny nursed by immunized females, regardless of their biological dam's immune status, and was also associated with the induction of neonatal suppressor T cells (Okamoto et al., 1989). Interestingly, T cell suppression of neonatal antibody responsiveness by specific maternal antibody transfer in the milk could be weakened by either high-dose antigen immunizations of suppressed offspring (Izuchi et al., 1985), or secondary immunization (Okamoto et al., 1989). Additionally, maternal antibodies transmitted to the offspring have been shown to shape the T cell repertoire of the neonate through anti-idiotypic interactions (Martinez et al., 1986). Passively-acquired maternal antibodies produced against various immunogenic stimuli may, therefore, play an important role in immunoregulation of subsequent homologous immune responses by the neonate.

Maternal Filarial Parasitism - Effects on Neonatal Immunity

Maternal parasitic infections could have a variety of effects on the developing fetus and/or neonate. The most obvious possibility would be transplacental or transmammary passage of the infectious agent to the progeny. Many species of human protozoan parasites, such as Plasmodium spp. and Toxoplasma gondii, can either infect the placenta itself, or pass across it initiating infection in the fetus (reviewed by Loke, 1982). Nematode parasites of veterinary importance such as Toxocara canis (Sprent, 1958), and both Ancylostoma caninum (Stone and Girardeau, 1966; 1968) and Strongyloides ransomi (Moncol and Batte, 1966) use transplacental and transmammary passage of infective larvae, respectively, as their primary modes of transmission. No reports exist, however, of an active filarial nematode infection being established in the fetus or neonate by transfer of infective L_3 or migrating L_4 lymphatic filarial parasites from the mother.

Microfilariae of various filarial nematode species have proven their ability to cross the placenta in both humans and experimental animals. In humans, microfilariae of Onchocerca volvulus (Brinkmann et al., 1976) and Wuchereria bancrofti (Blommfield et al., 1978; Rao et al., 1984) have been discovered in newborn children of microfilaremic mothers. No published reports are available concerning the occurrence of this phenomenon in Brugia malayi-infected mothers. Other natural host-parasite systems including

Dirofilaria immitis (Mantovani and Jackson, 1966) and D. repens (Mantovani, 1966) in dogs, and B. pahangi in cats (Kimmig, 1979) provide additional evidence for the transmission of microfilariae from mother to fetus. B. pahangi microfilariae have also been found in the fetal lung tissue of the white rat (Sucharit and Rongsriyam, 1980), which is a permissive host for this parasite. Two other artificial host-parasite systems using implanted adult female Acanthocheilonema viteae in rats (Haque and Capron, 1982) or mice (Storey et al., 1988) have provided some interesting insights into the potential for maternofetal microfilarial transfer to subsequently alter the homologous immune responses of their progeny.

The most dramatic effect of transplacental transfer of A. viteae microfilariae on neonatal immune responsiveness was observed in rats. Fischer rats are normally refractory to challenge infections initiated subcutaneously with A. viteae L_3. The progeny of microfilaremic, A. viteae-implanted mothers were, however, susceptible to challenge infections initiated 75 days after birth (Haque and Capron, 1982). This susceptibility to A. viteae challenge infection in the progeny of infected mothers was observed concurrently with neonatal microfilaremias, an in vitro blastogenic nonresponsiveness of neonate spleen cells to A. viteae antigens, and reduced in vitro splenocyte blastogenesis responses to the T cell mitogens phytohemagglutinin and

concanavalin A compared to the progeny of uninfected female rats. These data strongly suggest that susceptibility to A. viteae challenge infection in Fischer rats born of infected mothers was associated with an overall reduction of T cell responsiveness (Haque and Capron, 1982). A more recent study with A. viteae in mice, however, found that microfilaremic progeny of homologously implanted mothers were insusceptible to subcutaneous L_3 challenge infections like the progeny of uninfected mothers (Storey et al., 1988). Additionally, A. viteae antigen-stimulated splenocyte blastogenic responses of microfilaremic progeny of infected mothers were consistently higher than progeny of uninfected female mice. These two studies using different experimental models of A. viteae-implanted mothers suggest that either a tolerizing or sensitizing effect can occur on splenocyte blastogenic responses to parasite antigens as a result of transplacental transfer of microfilariae. Furthermore, the relative proliferative responsiveness of spleen cells from progeny exposed in utero to microfilariae, and who themselves express microfilaremia until well after birth, may reflect their relative susceptibility to subsequent L_3 challenge infection in these models. A question not addressed by either study is how susceptibility to challenge infection in these microfilaremic progeny may have changed following the clearance of maternally-derived microfilaremias. This particular question could have been

tested in the A. viteae-rat model. In this model utilized by Haque and Capron (1982), antigen-stimulated splenocyte blastogenesis responses of the progeny exposed to maternal filarial infections rose quite sharply at around 100 days of age concurrent with the clearance of circulating microfilariae derived from the mother. Challenge infections administered after the clearance of neonatal microfilaremias could have indicated whether the antigen-specific tolerance observed in blastogenic responses of spleen cells from these susceptible progeny was due solely to the presence of microfilariae, or to an adjustment of the T cell-mediated responsiveness to parasite antigens as a consequence of maternal filarial infections. Evidence from experimental studies in B. pahangi-infected jirds (Lammie and Katz, 1983a; 1983b; 1984a) implicates both a plastic-adherent cell suppression of in vitro mitogen-induced splenocyte blastogenesis, and a suppressor T cell responsible for the lack of in vitro splenocyte responses to A. viteae antigens observed in the microfilaremic progeny of homologously implanted mothers (Haque and Capron, 1982).

Two studies of W. bancrofti-infected mothers have indicated that fetal sensitization to filarial antigens in utero may also occur. Both IgM (Dissanayake et al., 1980) and IgE antibodies (Weil et al., 1983) to filarial antigens have been detected in the cordblood of babies born to mothers with bancroftian filariasis. Neither of these

immunoglobulin isotypes cross the unaltered human placenta into the fetal circulation. The evidence suggests, therefore, that either filarial antigens, or undetectable levels of microfilariae, crossed the placenta to sensitize the fetal immune system. Another study, however, failed to demonstrate measurable cordblood IgG, IgM, or IgE antibodies to microfilarial antigens of Loa loa (Van Hoegarden and Akué, 1986).

Evidence that in utero and/or neonatal exposure to maternal filarial infections can alter the responses of offspring to subsequent homologous challenge infections comes from two experimental studies in jirds. Female progeny of B. malayi-infected mothers displayed a higher percentage of patent infections compared to the infected female progeny of uninfected mothers (Schrater et al., 1983). Male offspring from B. pahangi-infected female jirds also displayed greater percent patency than males from uninfected females following homologous challenge infection (Klei et al., 1986). In addition, the infected male progeny of infected mothers exhibited less pathologic change in their lymphatics than infected male progeny of uninfected mothers following B. pahangi infections (Klei et al., 1986). Conversely, the progeny of A. viteae-implanted female mice discussed above (Storey et al., 1988) showed no alteration in expression of microfilaremias compared to the progeny of uninfected mothers following implantation of adult female A.

viteae into both sets of offspring. The results of the investigations in the progeny of female jirds infected with either B. malayi or B. pahangi suggest that progeny exposed to maternal filarial infections may not react as strongly to the homologous parasite, and thus allow the parasite to fully develop and more freely express microfilaremias following challenge infection. These findings are strikingly similar to comparisons of filarial infections in people born in areas endemic for filariasis and those previously unexposed people who immigrate into endemic areas.

The most revealing example of differential expression of microfilaremias in natives and immigrants comes from the studies of some 20,000 American servicemen who contracted lymphatic filarial infections while stationed in the South Pacific during World War II (Wartman, 1947). Symptoms of lymphatic filariasis in these Americans were evident within months of their arrival in an area endemic for filariasis, but fewer than 20 of the symptomatic servicemen had demonstrable microfilariae in their blood (Beaver, 1970). In communities within areas endemic for lymphatic filariasis, clinical symptoms can take from 2-10 years to develop in the infected indigenous population (Partono, 1987). The variance in clinical onset of filarial disease and subsequent expression of microfilaremias observed in native and non-native populations infected with lymphatic

filariae is purportedly due to differences in their relative immune responsiveness to the developing parasites (Partono, 1987). These apparent differences in immune responses to filarial parasitism have led others to hypothesize that in utero and neonatal exposure to maternal filarial infections may somehow alter the immune responsiveness of exposed progeny to subsequent homologous filarial infection; and thus partially account for the variety of clinical manifestations observed in human lymphatic filariasis (Otteson, 1980; 1984; Piessens et al., 1987).

Experimental Rationale

Results from the studies reviewed above have led to the hypothesis that in utero and/or neonatal exposure to filariae, filarial antigens, or maternal antifilarial immunity can alter the immune responses of progeny, and thus partially account for the wide spectrum of clinical manifestations observed in the human lymphatic filariases (Otteson, 1980; 1984; Piessens et al., 1987; Otteson, 1989). The purpose of this dissertation was to use the jird-Brugia pahangi model of lymphatic filariasis to critically test the hypothesis that exposure to maternal filariasis can alter the course of infection, lesion formation, and immune responses to subsequent homologous challenge infections.

The experiments included in Chapter 1 were designed to determine the source and characterisitics of IgG antibodies to B. pahangi antigens measured in the serum of progeny from infected female jirds. Additionally, experiments were conducted with uninfected progeny from infected and uninfected mothers to determine whether cellular immune and pathologic responses to parasite antigens were altered in progeny of infected mothers by the presence of these antifilarial antibodies.

Chapter 2 entails studies of the susceptibility, lymphatic lesion formation, and antibody responses to B. pahangi infections in young male jirds from uninfected mothers. The ability of young male jirds to support the development of B. pahangi was important information for subsequent experiments comparing the responses of progeny from infected and uninfected mothers to homologous infections.

Studies covered in Chapter 3 were designed to investigate the effects of exposure to maternal B. pahangi infections on the course of infection, lymphatic lesion development, and expression of immune responses following homologous challenge infection. These experiments were performed in an attempt to confirm earlier reports of reduced lymphatic lesion severity to challenge infections in maternally-exposed progeny (Klei et al., 1986), and to provide additional information regarding the immune

responsiveness of progeny from infected female jirds during homologous infections.

The Appendix contains significant, but unpublishable, information from two studies. Appendix A describes the results of experiments measuring *in vitro* cellular immune responses and *in vivo* granulomatous inflammatory reactions of progeny from *B. pahangi*-infected and uninfected female jirds following immunization with homologous adult worm antigens. Appendix B contains parasitological data pertaining to adult worm recoveries and microfilaremias from intraperitoneal *B. pahangi* infections established in breeding female jirds.

CHAPTER 1

Brugia pahangi: Circulating antibodies to adult worm antigens in uninfected progeny of homologously infected female jirds.

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ABSTRACT - Serum IgG antibody levels to adult B. pahangi antigens were measured in uninfected offspring from uninfected and B. pahangi-infected female jirds. Antibody titers to B. pahangi antigens in sera of offspring from infected females mimicked the maternal titer during the suckling period. Neonate titers peaked at 2 weeks of age at levels as high as 1:4100, then decreased to levels well below maternal titers by 8-12 weeks of age. Concurrent maternal and 2 week old neonate sera recognized identical B. pahangi antigens in Western blots. Spleen cells from 2 week old filariae-exposed and unexposed offspring failed to produce measurable antibody to B. pahangi in vitro. Progeny of uninfected mothers nursed by B. pahangi-infected females showed circulating IgG antibody titers to adult worm antigens similar to those of homologously reared offspring. Conversely, offspring born to B. pahangi-infected females and nursed by an uninfected female had no serum antibodies to B. pahangi antigens. Blastogenic responses of spleen cells to the mitogens PHA and PWM, and adult B. pahangi antigens, were not different between offspring groups. Mean areas of pulmonary granulomas induced by the intravenous inoculation of B. pahangi antigen-coated beads also did not differ between 4 and 8 week old progeny of uninfected or infected females. These results suggest that the circulating IgG antibodies to adult B. pahangi antigens demonstrated in offspring of infected female jirds are

maternally derived via the milk, and do not alter the cellular responses of uninfected offspring to B. pahangi antigens as measured by antigen-stimulated blastogenesis or pulmonary granulomatous inflammatory response.

Index Descriptors and Abbreviations: Nematode, *Brugia pahangi*, filariasis, passively-acquired immunity, jird, *Meriones unguiculatus*; Phytohemagglutinin A-P[PHA-P], Pokeweed mitogen[PWM], Immunoglobulin G[IgG], Intraperitoneal[IP], Enzyme-linked Immunosorbent Assay[ELISA], Third Stage Infective Larvae[L_3], 0.01M Phosphate-buffered Saline[PBS], 0.05% Tween 20 in PBS[PBST], Horseradish Peroxidase[HRP], PBS-Soluble Adult *B. pahangi* Antigenic Preparation[BpAg], Diethanolamine[DEA], Optical Density measured at 630nm[OD_{630}], Nitrocellulose paper[NCP], Cyanogen Bromide-Activated Sepharose 4B[CNBS].

INTRODUCTION

Many different immunologic and pathologic reactions have been described during human lymphatic filariasis. In general, however, patients fit into categories ranging from nonresponsive to hyperresponsive states of reactivity to infection with filarial parasites (Otteson, 1980; 1984). One hypothesis which could account for some of these variable clinical responses states that *in utero* and neonatal exposure to maternal filariasis alters subsequent homologous antiparasite responses of the progeny (Otteson, 1980; 1984; Piessens et al., 1987).

Evidence from animal models has demonstrated that exposure to maternal filariasis can alter parasitologic and pathologic events of subsequent homologous challenge infections in the progeny. A partial resistance to challenge infection with *B. pahangi* in some progeny of *Brugia*-infected cats has been reported (Denham and Suswillo, 1980). Conversely, Haque and Capron (1982) found that the offspring of *A. viteae*-implanted female Fischer rats were susceptible to homologous challenge infection unlike normal offspring. Schrater et al. (1983) have described the occurrence of patent *B. malayi* infections in female progeny of typically amicrofilaremic infected female jirds. Challenge infected male progeny of *B. pahangi*-infected female jirds have also been reported to exhibit more patent

infections than infected offspring from uninfected females, despite equal adult worm burdens (Klei et al., 1986). In addition, these infected male progeny of infected females produced fewer intralymphatic thrombi and demonstrated reduced overall lymphatic lesion severity compared to infected offspring from uninfected mothers.

The jird-B. pahangi model of lymphatic filariasis has demonstrated an immune-mediated component to intralymphatic lesion formation (Klei et al., 1982). The ability to quantitate intralymphatic lesions in infected male jirds makes it a useful system for the studying the effects of in utero and neonatal exposure to filariae on subsequent homologous infections, immune responses, and associated lesions. The purpose of this paper is: 1) to characterize the antibodies to adult B. pahangi antigens present in the sera of offspring exposed to homologous maternal filarial infections, and 2) to describe jird immune responses to B. pahangi antigens in filariae-exposed and unexposed offspring prior to challenge infection.

MATERIALS AND METHODS

L_3 of the filarial nematode Brugia pahangi were obtained by crushing cold-anesthetized, infected Aedes aegypti 11 days after ingestion of patent jird blood. L_3 were allowed to settle through cheesecloth into a Baermann apparatus containing RPMI 1640 medium (Grand Island Biological Co., Grand Island, NY) at 37°C supplemented with Hepes buffer (25mM), penicillin (200U/ml), streptomycin (200ug/ml), and amphotericin B (0.5ug/ml). Doses of 100-200 L_3 were counted with the aid of a stereo microscope and injected IP into female jirds in <1.0ml of supplemented RPMI 1640.

Inbred male and female jirds (Meriones unguiculatus) used to establish monogamous breeding pairs were obtained commercially (Tumblebrook Farms, West Brookfield, MA), and maintained on Purina lab chow and water ad lib. Offspring from B. pahangi-infected mothers were obtained from female jirds inoculated IP at approximately 15 weeks of age with 100 B. pahangi L_3. Offspring were accumulated from age matched infected (Group 1) and uninfected female jirds (Group 2) once maternal B. pahangi infections had reached 100 days duration. All breeder female jirds were necropsied 11 months after infection of Group 1 mothers for assessment of adult worm burdens, and immunologic reactivity of both maternal groups to BpAg.

All immunologic assays utilized BpAg prepared as described previously (Klei et al., 1982). Titers of IgG antibodies to BpAg in maternal, neonatal, and juvenile sera were measured using an ELISA. Immulon I flat-bottomed 96-well polystyrene microtiter plates (Dynatech Laboratories, Inc., Alexandria, VA) were coated overnight at 4°C with 50ul/well of a 0.02M carbonate buffer, pH 9.6, containing 15ug/ml BpAg. Plates were washed 3X for 5 minutes each with 0.2ml/well PBST before serial two-fold dilutions of maternal and neonatal sera from Groups 1 and 2 and pooled normal jird sera were performed in each plate. Plates were washed as above after a 20 minute incubation at 20°C and jird IgG antibodies to BpAg were detected using a 1:2000 dilution in PBST of rabbit antiserum raised against jird IgG purified by Protein A affinity chromatography techniques previously described (Miller and Stone, 1978). Plates were washed after a 45 minute incubation at 20°C and 0.05ml/well of a 1:1000 dilution of antirabbit IgG conjugated with HRP (Kirkegaard and Perry Laboratories, Inc., Gaithersburg, MD) in PBST was added. Plates were again incubated for 45 minutes at 20°C and washed as above. Substrate solution consisting of 0.005M 5-aminosalicylic acid, pH 5.7, and 0.0005% H_2O_2 was added at 0.1ml/well, and the reaction stopped with 1N NaOH after 20 minutes. The OD_{630} of normal and test serum dilutions was measured, and antibody titers expressed as the $\log_2$ dilution of test serum generating an

OD_{630} value >2X the OD_{630} value of the lowest normal serum dilution.

Serum antibodies to B. pahangi antigens in infected female jirds and their offspring were qualitatively assessed by Western immunoblot. Parasite antigens were separated by sodium dodecyl sulfate polyacrylamide gel electrophoresis using a 7.5-20% acrylamide gradient separating gel and methods previously described (Laemmli, 1970). Separated BpAg and prestained molecular weight markers (Bethesda Research Laboratories, Bethesda, MD) were transblotted from the gel to 0.22um NCP (Schleicher and Schuell,Inc., Keene, NH), and 3mm strips removed for immunoblotting after staining with 0.5% Ponceau S dye. NCP strips were blocked for 30 minutes at 20°C with a solution of 5% w/v nonfat dry milk and 0.3% Tween 20 in PBS. Normal and test sera were diluted 1:100 in PBST and incubated with individual strips for 90 minutes at 20°C. Rabbit antijird IgG and goat antirabbit Ig conjugated with HRP (Pel-Freez Biologicals, Rogers, AR) were diluted separately in PBST at 1:2000 and 1:1000, respectively, and incubated with the NCP strips for 90 minutes each at 20°C. Strips were washed 3X for 10 minutes each between antibody incubation steps, and prior to the addition of a substrate solution consisting of 35mg 4-chloro-1-naphthol in 50ml of 0.05M Tris-HCl, pH 6.8, plus 10ml absolute methanol and 30ul of a 30% H_2O_2 solution. The substrate reaction was stopped with distilled water.

Pulmonary inflammatory responses of 4- and 8-week old offspring from infected and uninfected female jirds were measured by the methods of Klei et al. (1988) around embolized CNBS (Sigma Chemical Co., St. Louis, MO) coupled with either BpAg, or DEA as a control for foreign body inflammatory reactivity. Granuloma areas are expressed in um^2.

Lymphocyte blast transformation assays were performed by modified methods of Prier and Lammie (1988). Spleens from 4 and 8 week old progeny of B. pahangi-infected and uninfected mothers were reduced to single cell suspensions by mincing in RPMI 1640 lacking 2-mercaptoethanol and supplemented with Hepes buffer (25mM), L-Gln (2mM), penicillin (100U/ml), streptomycin (100ug/ml), amphotericin B (0.25ug/ml), and 2% fetal calf serum. Washed cells were counted in Turk's solution and viability estimated by Trypan blue exclusion. Cells were cultured in triplicate at 5.0 x 10^5 cells/well in 0.2ml/well and either unstimulated, or stimulated by a 1:500 dilution of PHA-P (Sigma Chemical Co., St. Louis, MO), or a 1:100 dilution of PWM (Grand Island Biological Co., Grand Island, N.Y.) in 96-well flat-bottomed plates, or 20ug/ml BpAg in round-bottomed plates. Data are expressed as either net counts/minute obtained by subtracting the counts/minute of unstimulated cultures from the autologous mitogen or antigen stimulated cultures, or as a stimulation index calculated by dividing the counts/minute

obtained from stimulated cultures by the counts/minute obtained from autologous unstimulated cultures.

In vitro synthesis of antibodies to BpAg by spleen cells derived from 2 week old filariae-exposed and unexposed neonate jirds were accomplished in separate assays by the methods of Prier and Lammie (1988). Supernatant antibody levels to BpAg were measured by ELISA after a 10 day culture period.

Rocket electrophoresis was performed by the methods of Barta et al. (1984) using a gel consisting of 1:40 diluted rabbit antijird IgG in 1% Low EEO agarose (Sigma Chemical Co.,St. Louis, MO) on 10x15cm sheets of Gelbond™ (Bioproducts, Rockland, ME). Undiluted culture supernatants, or serum diluted 1:4 in 0.05M Veronal buffer, pH 8.6, were electrophoresed for 2 hours at 20°C. Precipitated rockets were visualized by staining with 0.2% Coomassie blue after removal of unbound proteins.

Statistical analysis of antibody titers to BpAg in serum and culture supernatants were done using the Student's t-test. Lymphocyte blastogenesis data were analyzed by analysis of variance and Duncan's multiple range test. The Kruskal-Wallis test was used to analyze pulmonary granuloma areas, and comparison of group means accomplished using a nonparametric equivalent of the Tukey test.

RESULTS

The mean and standard deviation of adult B. pahangi recovered from Group 1 female jirds at 11 months postinfection was 8.3 ± 12.0, with a range of 0-53. Adult worms were typically located loose in the peritoneal cavity, but intralymphatic worms were found in 9/20 infected females. Microfilaremias were detected in 75% (15/20) of Group 1 females at some time during these studies at levels ranging from 1-212mf/20ul of whole blood. All Group 1 females with 0 detectable adult parasites at necropsy (n=3) had microfilariae in their spleens. Log_2 serum antibody titers to BpAg in Group 1 breeding females were similar at 90 days postinfection ($\overline{X}$±sd=10.3 ± 1.0) and at necropsy ($\overline{X}$±sd=9.8 ± 1.2). Group 2 females, negative for antibody initially, developed a background titer of 1 against BpAg measured at necropsy. Not surprisingly, pulmonary inflammatory reactions of Group 1 females to BpAg-coated CNBS were greater than those of Group 2, and produced significantly (α=0.0) larger granuloma areas of 9700um^2 versus 5800um^2. Foreign body inflammatory reactions to DEA were similar for both female groups at approximately 4800um^2.

Group 1 offspring displayed consistent patterns of serum IgG antibody titers to BpAg, in ELISA, as shown in Figure 1. Group 1 neonate titers increased from a $\overline{X}$ log_2

titer of approximately 5 just after birth to a peak of 12 at 2 weeks of age. Peak neonatal titers were equivalent to the levels of their respective mothers. Juvenile titers decreased through weaning at 4 weeks of age to levels well below maternal titers by 8-12 weeks after birth. Offspring from uninfected mothers (Group 2) showed no reactivity to BpAg in ELISA, despite a background maternal titer of 1.

Rocket immunoelectrophoresis of these concurrent maternal and neonatal sera indicated that neonatal jird IgG levels were lower than their respective maternal levels (Groups 1 and 2) at birth. Neonatal serum IgG concentrations increased to levels equivalent to that of maternal serum by approximately 14 days after birth in both Groups 1 and 2.

Western immunoblots reacting Group 1 maternal and 2 week old neonate sera against BpAg (Figure 2) revealed neonatal binding patterns that were identical to maternal patterns. Group 1 neonatal serum antibody reaction intensity to BpAg in Western blots decreased noticeably compared to maternal levels after the progeny were 8-12 weeks old corresponding to a drop in ELISA titers at this time.

The source of the Group 1 neonatal serum IgG antibodies to B. pahangi was investigated by measuring the in vitro antibody production of spleen cells derived from 2 week old Group 1 and 2 jirds during the peak serum antibody titers

observed in Group 1 offspring. The splenocyte responses of two litters of Group 1 neonates (A and B) and one Group 2 litter, as well as cells from infected and uninfected adult female jirds, to BpAg stimulation after 10 days in culture are shown in Table I. BpAg concentrations ranging from 0-500ng/ml, a range shown by Prier and Lammie (1988) to generate an *in vitro* antibody response curve, failed to stimulate production of measurable IgG antibody in either Group 1 or 2 splenocyte cultures, despite the high autologous serologic titers in Group 1 progeny. This is demonstrated by OD_{630} values of approximately 0.02 or less from Group 1 and 2 culture supernatants. Negative control splenocytes also produced no supernatant IgG antibodies to BpAg. Spleen cells from female jirds infected with *B. pahangi* 30-45 days previously, however, showed high levels of supernatant antibodies to BpAg at all concentrations of stimulating antigen. All culture supernatants produced rocket electrophoresis patterns of equivalent size against rabbit antijird IgG indicating secretion of similar levels of immunoglobulin by all cultures.

The transfer of maternal IgG antibodies to BpAg in the milk was tested by allowing a Group 1 litter to nurse a Group 2 female and a Group 2 litter to nurse a Group 1 female. The kinetics of serum IgG antibody titers to BpAg in Group 2 offspring suckling a Group 1 female jird (Figure 3) were comparable to those seen in homologously reared

Group 1 offspring. Western immunoblots of concurrent Group 2 neonate and Group 1 maternal sera again indicated identical antigen recognition patterns (data not shown). Group 1 progeny reared by a Group 2 female showed no serum IgG antibody titers to BpAg and no Western blot reactivity.

In vitro blastogenesis and in vivo pulmonary granuloma assays were used to compare the cellular immune and inflammatory reactions of uninfected, filariae-exposed and unexposed progeny to BpAg. Significant differences were not found in 2 experiments comparing splenocyte blastogenesis responses of Group 1 and 2 progeny to PHA, PWM, or BpAg stimulation. Splenocyte stimulation indices to PHA demonstrated means ranging from 34-38 and 9-52 in 4 week old progeny, and 32-36 and 12-30 in 8 week old progeny of Group 1 and 2 females, respectively. Responses to PWM were similarly variable in both groups of young in different experiments. BpAg produced mean stimulation indices of 1.5-4.0 (net counts/minute$<10^3$) in spleen cells of both ages of Group 1 and 2 offspring. Cellular inflammatory responses measured in the lungs of 4 and 8 week old jirds from the same 2 experiments showed no significant differences in granulomatous reactivity to BpAg-coated CNBS in either age of Group 1 and 2 offspring. Granuloma areas around BpAg-CNBS measured 6300-8200um^2 in all offspring groups. DEA-CNBS elicited granuloma areas of 6000-7000um^2 in all offspring groups. Antibody titers to BpAg were significantly

lower in Group 1 offspring at 8 versus 4 weeks of age, but granuloma areas around BpAg-coated CNBS were not significantly affected by this change.

DISCUSSION

Human fetal sensitization to antigens of W. bancrofti has been measured by the presence of either IgM (Dissanayake et al., 1980) or IgE (Weil et al., 1983) antifilarial antibodies in cord blood. The presence of serum antibodies to BpAg in the offspring of B. pahangi-infected female jirds may be a result of either fetal/neonatal sensitization leading to an active neonatal immune response, and/or the passive transfer of maternal antibody. Parasitologic, pathologic, and immunologic observations of B. pahangi-infected mothers of Group 1 progeny indicate that these offspring were exposed in utero and postpartum to maternal filarial infections and associated immune responses. Results from in vitro antibody production assays, however, indicate that splenocytes of neonates exposed to maternal filariasis both in utero and postpartum do not produce detectable amounts of antibody to BpAg. This lack of measurable in vitro antibody from neonatal spleen cells is not due to unsuitable culture conditions since rocket electrophoresis patterns of all culture supernatants demonstrated equivalent immunoglobulin production. Group 1 neonatal splenocytes, therefore, do not produce measurable antifilarial antibody at 2 weeks of age despite the presence of high circulating levels of anti-B. pahangi antibody. These results support the hypotheses that these antibodies

are of maternal origin, and that unlike human fetuses, jirds may either not be exposed to filarial antigens *in utero*, or if exposed are incapable of responding to these antigens at this age.

Studies from humans and animal models have proven that microfilariae can cross the placenta, an occurrence possibly leading to a state of immunologic tolerance or sensitization in the neonate. Humans can be exposed *in utero* to the microfilariae of *Onchocerca volvulus* (Brinkmann et al., 1976), and *Wuchereria bancrofti* (Bloomfield et al., 1978; Rao et al., 1984). The presence of fetal microfilariae, or microfilaremias, has also been documented using *Dipetalonema viteae* (=*Acanthocheilonema viteae*) in jirds (Geigy, 1976), rats (Haque and Capron, 1982), and mice (Storey et al., 1988). These latter two studies, using implanted adult *D. viteae* in rats (Haque and Capron, 1982) or mice (Storey et al., 1988), have suggested that the transplacental transfer of microfilariae can either tolerize or sensitize neonatal splenocytes, respectively, as measured by blastogenesis responses to parasite antigens. Microfilariae of *Brugia pahangi* have been described in the fetal lungs of white rats (Sucharit and Rongsriyam, 1980), and cats (Kimmig, 1979). However, *Brugia pahangi* microfilariae have not been reported to cross the placenta of *Meriones unguiculatus*, and attempts to demonstrate either neonatal microfilaremia, or fetal tissue microfilariae, have been unsuccessful (Bosshardt and

Klei, unpublished observations). The lack of evidence for transplacental transfer of B. pahangi microfilariae in the jird could explain the lack of in utero sensitization observed in this model.

Onchocerca volvulus antigens have been detected in the breast milk of infected mothers (Petralanda et al., 1988) indicating another route for the induction of either neonatal sensitization or tolerance (Gill, 1973; Richman et al., 1978; Kleinman and Walker, 1984) to ingested filarial antigens. Attempts to measure a B. pahangi antigen containing a phosphorylcholine epitope in the sera of Group 1 neonatal and juvenile jirds at a time when it was demonstrable in maternal sera were unsuccessful (Weil et al., 1990). These results provide a potential explanation for the lack of in utero/neonatal sensitization to this filarial antigen. The occurrence of filarial antigens smaller than this ≥100kd PC-containing antigen has not been tested, leaving the question of in utero/neonatal sensitization to smaller antigenic molecules in this model unanswered.

Passive transfer of maternal antibodies is the alternate explanation for high titers of serum IgG antibodies to BpAg in the progeny of B. pahangi-infected female jirds. Quantitative and qualitative reactivity of Group 1 neonatal serum antibodies to BpAg varied between litters from different mothers as a function of their

respective maternal serum antibody reactivity. The antibodies in Group 1 neonatal sera were shown by Western blot to have the identical parasite antigen recognition profile as their respective maternal sera. Group 2 offspring fostered by a Group 1 female also demonstrated high titers of serum IgG antibody to BpAg, while Group 1 progeny fostered by Group 2 females did not. The heterologous maternal antibodies measured in Group 2 offspring in the latter instance also produced identical maternal and neonatal Western blot profiles to BpAg. Furthermore, serum immunoglobulin levels of offspring from both maternal sources were almost equivalent to that of their mother at a time when the serum antibody titers of Group 1 progeny reached maternal levels. These observations strongly suggest that B. pahangi-infected female jirds passively transferred antibodies to BpAg to their offspring through the milk, as reviewed by Brambell (1970) for mice.

Maternal antibodies in mice have been shown, through idiotypic network interactions, to select for T-cell repertoires early in neonatal life (Martinez et al., 1986). In another chronic helminthic disease of humans, schistosomiasis mansoni, idiotypic regulation of T cell responsiveness has been reported in both the murine model (Powell and Colley, 1987), and humans (Montesano et al., 1989; Colley et al., 1989). The immunoregulatory idiotypes in human sera directed against soluble egg antigens were

identified more frequently in asymptomatic or hepatointestinal schistosomiasis patients than in acute or hepatosplenic patients suggesting an idiotypic association with a reduced state of disease (Montesano et al., 1989). Furthermore, peripheral blood mononuclear cells from schistosomiasis patients exposed to these idiotypic antibodies to egg antigens demonstrated the ability to suppress *in vitro* granuloma formation by syngeneic peripheral blood mononuclear cells to homologous antigens (Doughty et al., 1987; Colley et al., 1989). In the present study, BpAg-stimulated spleen cells from both offspring groups at both ages did not respond in blastogenesis assays. These data do not indicate a stimulatory effect of either maternal antibodies or filarial antigens on neonatal cellular responsiveness to BpAg in uninfected progeny of Group 1 female jirds as measured by *in vitro* splenocyte blast transformation assays. The variability in quantity and specificity of maternally-derived antibodies to BpAg may partially explain this lack of measurable effect on neonatal cellular responses, although immunoregulatory events necessary to significantly alter the blastogenic responses of Group 1 offspring to homologous parasite antigens following *in utero*/neonatal exposure to maternal filariasis may only become evident after exposure of these offspring to challenge infection. A correlation has been shown between the size of pulmonary granulomas around BpAg-coated beads

and the severity of lymphatic lesions in B. pahangi-infected jirds (Klei et al., 1988; 1990). Lewert and Mandlowitz (1969), using intravenously inoculated S. mansoni eggs, measured decreased granuloma sizes around embolized eggs in the lungs of mice born to S. mansoni-infected mothers compared to progeny of uninfected mothers suggesting an alteration (tolerization) of delayed hypersensitivity responses of the progeny due to exposure to maternal schistosomiasis. Camus et al. (1976) described increased delayed hypersensitivity reactions of children from S. mansoni-infected mothers compared to controls by skin tests using homologous worm antigens. Comparisons of pulmonary granulomatous reactivity of uninfected 4 and 8 week old Group 1 and 2 offspring to BpAg from 2 separate experiments did not suggest any alterations of Group 1 granulomatous responses due to maternal filariasis. These data lead to the conclusion that the size of inflammatory responses to BpAg-CNBS in the lungs of Group 1 offspring displaying high serum antibody titers to B. pahangi were not measurably affected by in utero or neonatal sensitization, or by maternal idiotypic regulation to filarial antigens present in the BpAg used for these assays. This lack of measurable effects of exposure to maternal filariasis on the cellular responses of the progeny may again be due in part to the variabilities observed in maternal antibodies transferred to these progeny. It is also possible that pulmonary

inflammatory responses of filariae-exposed offspring during subsequent homologous challenge infection may differ significantly from those of similarly infected unexposed offspring, as is implied by the reduced lymphatic lesion severity reported previously in this model (Klei et al., 1986), but that these alterations do not occur in the responses of uninfected progeny to antigens alone.

In summary, B. pahangi-infected female jirds supply their nursing progeny with variable amounts and specificities of IgG antibodies to homologous parasite antigens. Neither the presence of these anti-B. pahangi maternal antibodies in the serum of their progeny, nor gestational exposure to maternal filariasis led to specific immunologic sensitization of these offspring to the parasite compared to the progeny of uninfected mothers as measured by parasite antigen-stimulated in vitro antibody production, lymphocyte blastogenesis, or in vivo pulmonary granuloma formation. The lack of effect of maternal filariasis on homologous neonatal jird immune responses prior to challenge infection reported here may be partially due to the lack of transplacental transfer of B. pahangi microfilariae or antigens, the variable quantity and/or antigenic specificity of the passively-transferred maternal antibodies, or the lack of the appropriate filarial antigens in the BpAg used in our immunologic assays. However, the findings of Montesano et al. (1989) of specific immunoregulatory

idiotypes against schistosome egg antigens that are associated with less severe clinical forms of human schistosomiasis suggests a possible scenario whereby maternal antifilarial antibodies (idiotypes) from asymptomatic mothers could regulate the responses of their progeny and, upon homologous infection, yield a clinical form of lymphatic filariasis similar to that of the mother. Our lack of evidence in these studies for maternally altered neonatal responses to B. pahangi antigens in filariae-exposed progeny prior to homologous exposure does not preclude the altered responsiveness to homologous infections in offspring of B. pahangi-infected females reported previously (Klei et al., 1986). Instead, it underscores the importance of further investigations into the mechanisms by which maternal filariasis may affect the responses of the progeny after subsequent exposure to homologous infection.

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Table I. IgG antibody levels to adult Brugia pahangi antigens in serum and spleen cell culture supernatants derived from 2 week-old progeny of uninfected and infected mothers.

Maternal Source	N	Serum Titer	BpAg Stimulation (ng/ml) 0.0	0.5	1.0	10.0	100	500
Infected A	6	7.7	0.8*	0.8	1.3	0.9	1.1	1.4
Infected B	4	8.5	1.0	1.2	1.4	0.9	1.1	1.5
Uninfected	5	2.6	0.2	0.0	0.9	0.1	0.8	1.3
Pos. Control**	1	6.5	33.2	33.3	33.4	37.2	39.1	36.5
Neg. Control+	1	0.0	2.5	2.3	2.2	1.2	1.3	2.1

Footnotes for Table I.

* $\overline{X}$ OD_{630} (x 10^{-2}) generated in ELISA by undiluted supernatants from triplicate spleen cell cultures.

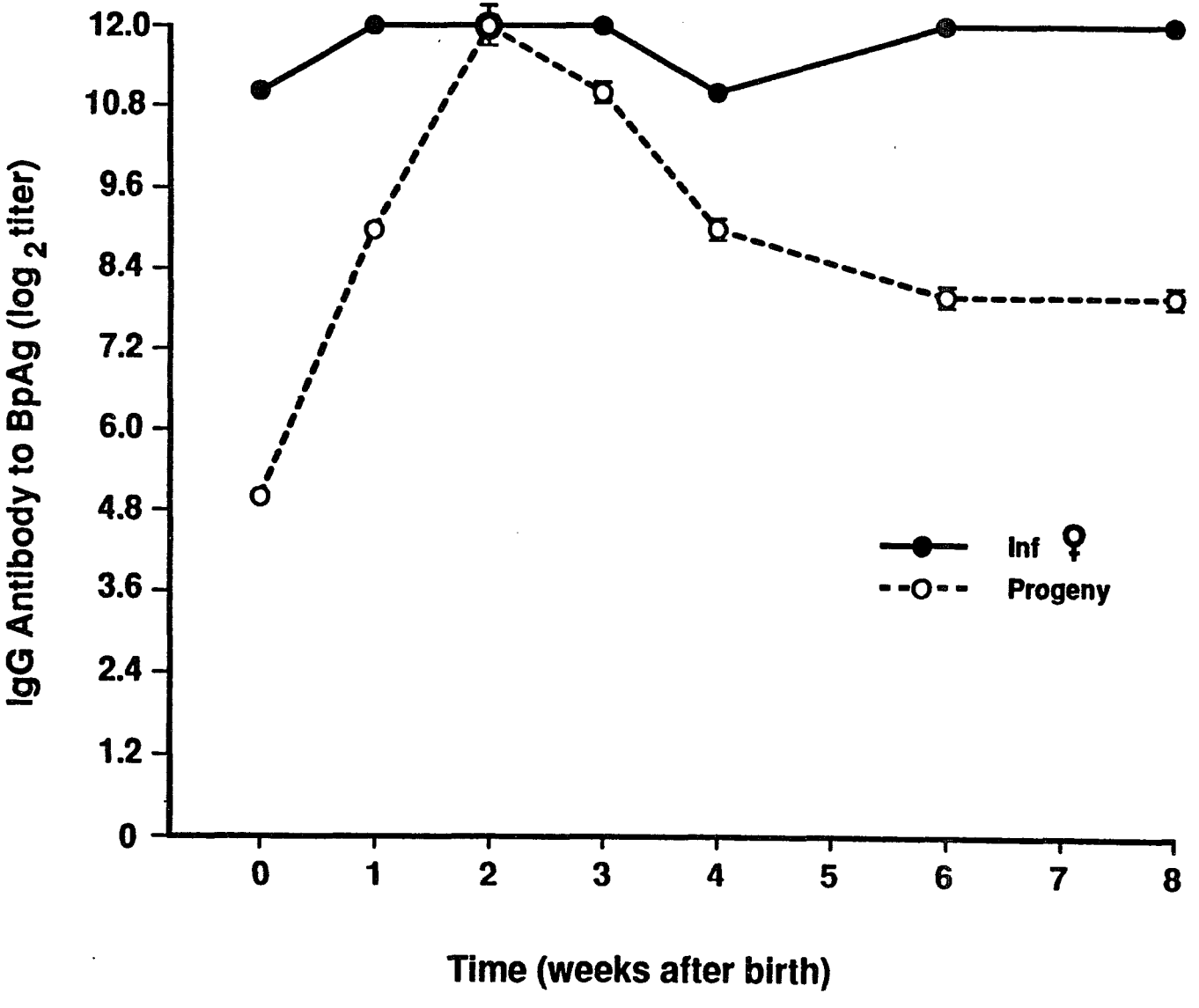
**Positive control consisted of splenocytes from an adult female jird infected with B. pahangi for 30-45 days.

†Negative control consisted of splenocytes from an uninfected adult female jird.

Figure 1. ELISA antibody titers to adult *Brugia pahangi* antigens measured in the sera of a *B. pahangi*-infected female jird (●——●) and her uninfected litter (n=3) from 1 day to 8 weeks of age (○·····○). Standard deviation bars are present except for data collected from single animals on Weeks 0 and 1, and maternal titers.

Figure 2. Western blot serologic reactions to adult *Brugia pahangi* antigens of 3 *B. pahangi*-infected females and their respective litters at 2 weeks of neonate age. Lanes A, E, and I represent maternal reactions, while Lanes B-D, F-H, and J-L are the reactions of individual offspring from their corresponding litters, respectively. Lane M shows the reactivity of an uninfected female, and Lane N represents the reactions of pooled offspring sera (n=6) from this uninfected female. Lane O shows a negative control reaction.

Figure 3. ELISA antibody titers to adult *Brugia pahangi* antigens in the sera of a litter (n=6) born to an uninfected mother (□---□) nursed by a *B. pahangi*-infected mother (●——●), and a litter (n=6) born to a *B. pahangi*-infected female (○——○) nursed by an uninfected mother (■---■), from 1 day to 8 weeks of age. Standard deviation bars are present except for data collected from single animals on Weeks 0 and 1, and maternal titers.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IgG Antibody to BpAg (log 2 titer)
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Time (weeks after birth)
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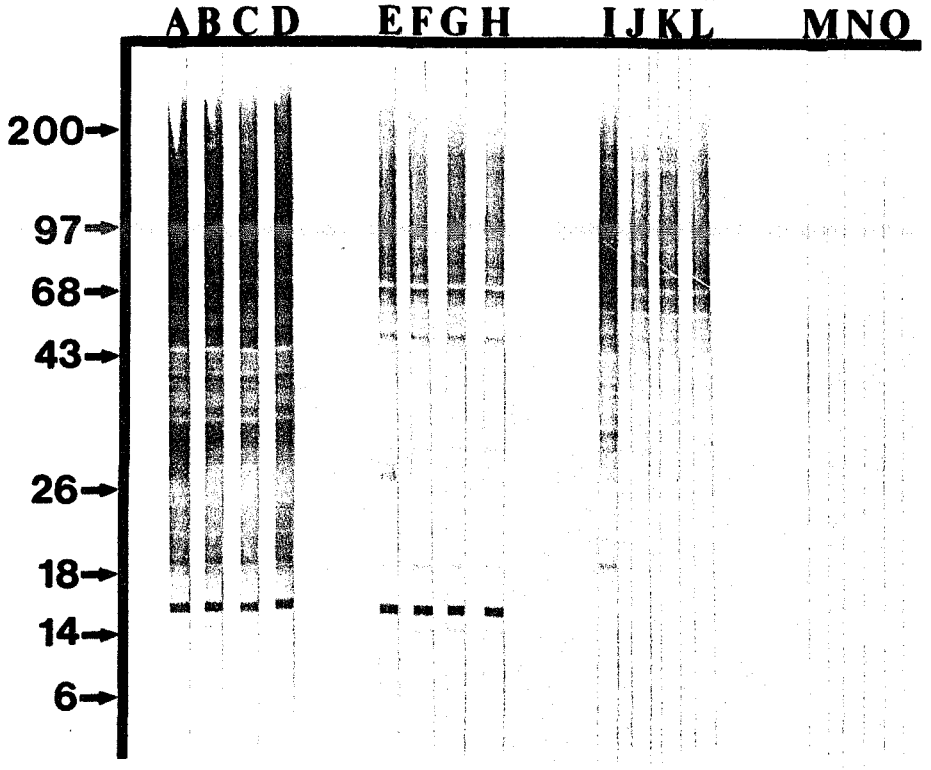
Progeny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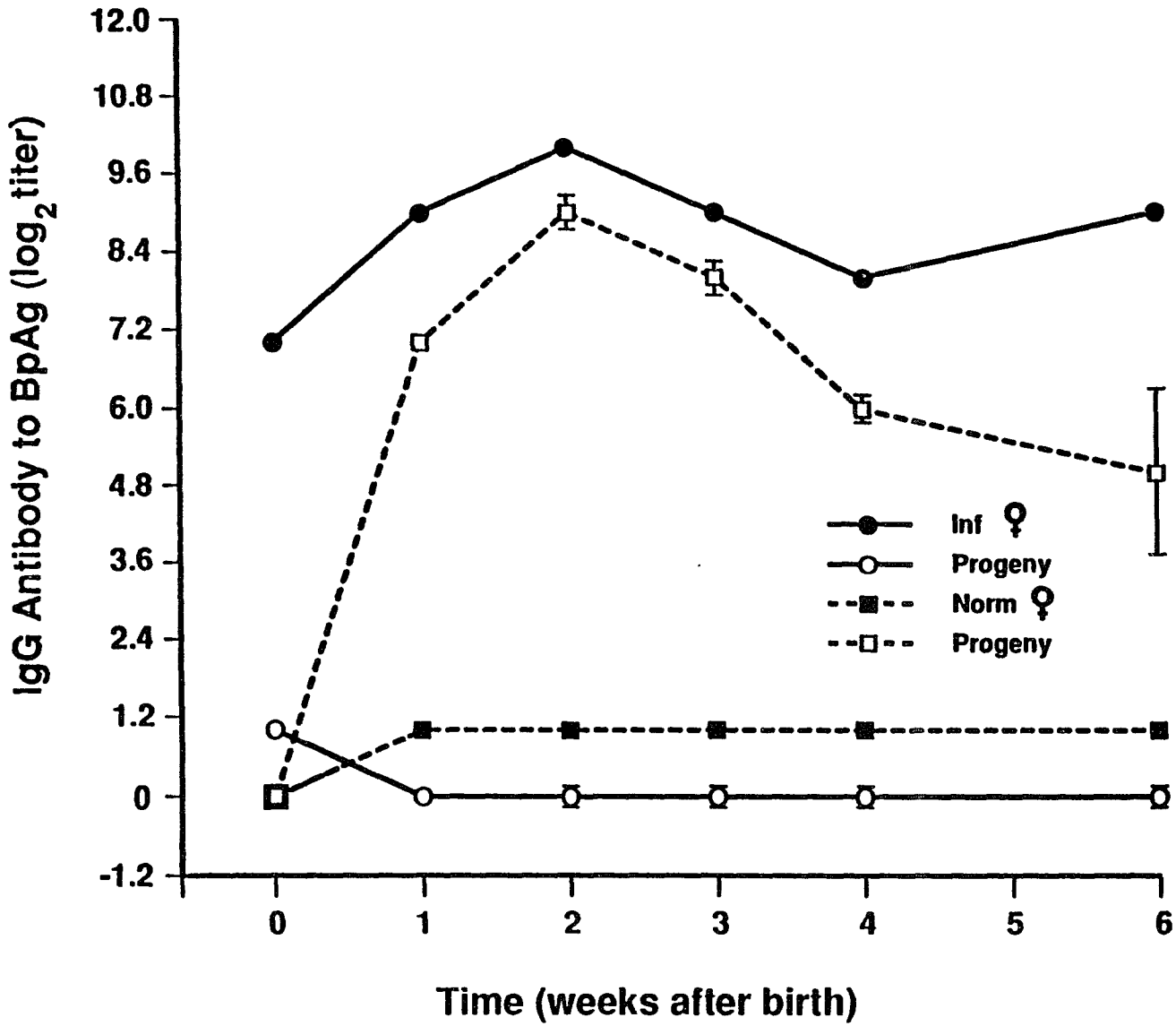
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IgG Antibody to BpAg (log2 titer)
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Time (weeks after birth)
Inf ♀
Progeny
Norm ♀
Progeny

CHAPTER 2

Altered Adult Worm Location and Antibody Responses in Young Male Jirds Infected With *Brugia pahangi*

Stephen C. Bosshardt, Sharon U. Coleman, Donald L. Thompson, Jr., and Thomas R. Klei

As submitted to the Journal of Parasitology

ABSTRACT: Male jirds (Meriones unguiculatus) were inoculated subcutaneously with 100 Brugia pahangi L_3 each at 2, 6, 10, and 15 wk of age to compare their susceptibility, pathologic reactivity, and antibody responsiveness to infection. No significant differences in susceptibility were measured between the 4 age groups. Adult worm recoveries (mean ± SD) ranged from 24.1 ± 15.1 to 36.4 ± 13.9 at 60 days postinfection. Jirds infected at 2 wk of age had significantly fewer ($\alpha \leq 0.025$) testicular and intralymphatic worms, and intralymphatic thrombi, than all other age groups. The ratio of intralymphatic thrombi per intralymphatic worm was not different between age groups. Serum IgG antibody responses to adult B. pahangi antigens in jirds infected at 2 wk of age were significantly reduced compared to all other age groups at 4 and 6 wk postinfection. These data indicate no differences in susceptibility or pathologic responsiveness to B. pahangi infection in male jirds as young as 2 wk old, despite altered adult worm location and reduced antibody responses to soluble antigens in these animals.

INDEX DESCRIPTORS: Nematoda, Filarioidea, *Brugia pahangi*, Lymphatic filariasis, *Meriones unguiculatus*, Jird, Age-related susceptibility.

Differences in age-related susceptibility and immune reactivity in a permissive host have unknown effects on the severity of the subsequent immunopathologic reactions of the host to filarial parasites. These potentially variable immunological and pathological responses by the infected host could partially explain the broad spectrum of clinical manifestations observed in human lymphatic filariasis (Otteson, 1980; 1984). The purpose of this study was to use the jird-B. pahangi model for lymphatic filariasis to directly test age-related susceptibility to infection, and to measure any differences in subsequent immunologic and pathologic host responses.

Groups of 10 male jirds were individually inoculated s.c. at 2, 6, 10, or 15 wk of age with 100 B. pahangi infective larvae (L_3) each as previously described (Klei et al., 1987). Infected jirds, and 1-2 uninfected age-matched control animals per age group, were bled prior to infection and at wk 2, 3, 4, 5, 6, and 8 PI for measurement of both total and differential white blood cell numbers. Concurrent serum samples were collected for the determination of immunoglobulin G (IgG) antibody levels against B. pahangi antigens by enzyme-linked immunosorbent assay (ELISA). Serum antibody levels are expressed in ELISA units measured from a standard curve generated with known positive pooled sera included in each assay. Necropsies were performed 60 days after infection by previously described methods (Klei

et al., 1982). Data collected at necropsy included adult worm burdens and location, microfilaremias, and the enumeration of intralymphatic lesions within infected jirds. Statistical analyses of nonparametric data (adult worm recoveries, numbers of intralymphatic thrombi, and testosterone levels) were performed using the Kruskal-Wallis test and the mean ranks of groups were compared using a nonparametric equivalent of the Student-Newman-Keuls test (Zar, 1984). Antibody responses and white blood cell counts were compared by analysis of variance and the Tukey test. Data are expressed as the mean ± the SD. Significant differences between group means were assessed at a 95% confidence level.

Adult B. pahangi recoveries did not significantly differ between the 4 age groups of jirds. However, jirds infected at 2 wk of age displayed altered adult worm distribution compared to all other age groups (Table I). Significantly fewer worms were recovered from intralymphatic sites (including testes) in jirds infected at 2 wk of age compared to infections in older jirds.

Testosterone levels were measured in the sera of all jirds on the day of infection by previously described methods (Gay and Kerlan, 1978) in an attempt to correlate the relative levels of sex hormones and susceptibility to infection. Testosterone levels in 2-wk old jirds (0.15 ± 0.17 ng/ml) were significantly less than 10- (5.35 ± 3.74

ng/ml) and 15-wk old jirds (3.03 ± 1.96 ng/ml), but not 6-wk old jirds (1.69 ± 2.33 ng/ml). The Spearman rank correlation coefficient of initial testosterone levels and worm burdens at necropsy was, not surprisingly, insignificant ($r_s = 0.198$; $\alpha(2) > 0.2$).

Jirds infected when 2 wk old developed significantly fewer intralymphatic thrombi (9.0 ± 11.9) compared to those infected at either 6 wk (30.4 ± 16.4), 10 wk (30.3 ± 8.0), or 15 wk (36.2 ± 13.4) of age. The ratio of numbers of intralymphatic thrombi per intralymphatic worm did not significantly differ between the 4 age groups with means ranging from 1.20 ± 0.59 to 1.38 ± 0.88.

The preinfection white blood cell deficit observed in 2- (7120 ± 1458/ul) versus 6- (12,520 ± 2118/ul), 10- (14,810 ± 1831/ul), and 15-wk old jirds (16,610 ± 1850/ul) was due to significantly reduced numbers of blood lymphocytes. Levels of eosinophils in the blood of infected jirds rose above their respective uninfected controls at 4-5 wk postinfection, but this difference was significant only in the oldest age group. No other significant differences in numbers of circulating leukocytes were noted between groups of infected or uninfected jirds.

Serum antibody responses to infection with B. pahangi were also found to be different between age groups (Table II). Circulating IgG antibodies to soluble adult B. pahangi antigens were significantly reduced in jirds infected at 2

wk of age compared to the other 3 age groups at 4 and 6 wk PI. Jirds infected at 2 wk of age also demonstrated the lowest level of serum antibodies to adult worm antigens at necropsy, but this reduction was significant only when compared to jirds infected at 6 or 10 wk of age.

Two separate studies of Brugia pahangi infections in mice have shown that younger mice are more susceptible to infection than older mice (Furman and Ash, 1983; Nakanishi et al., 1990). Other experiments have suggested that jirds are less susceptible to B. pahangi infections at 2 weeks than at 4 weeks of age (Bosshardt et al., in preparation). The results of the latter study, however, were derived from experimental infections initiated with different batches of L_3, and direct comparisons of worm recovery data cannot be accurately made. Data from the current study indicate that susceptibility to B. pahangi infection, as measured by adult worm recoveries, is not significantly affected by host age. The trend of both the previous and current studies, however, suggests that younger male jirds may be less susceptible to B. pahangi infection than older males.

Sex hormones are known to affect the course of parasitism in nematode infections of both humans and experimental animals (Alexander and Stimson, 1988). Higher levels of androgens, such as testosterone, have been linked to increased susceptibility to i.p. B. pahangi infections in mice (Nakanishi et al., 1989; 1990). Testosterone may also

be important for the development of B. pahangi in the jird. Preinfection levels of testosterone measured in the sera of all jirds indicated that 2-wk old jirds had significantly less circulating testosterone compared to 10- and 15-wk old jirds. Worm recoveries, however, did not reflect these differences. Our data suggest that serum testosterone levels do not affect jird susceptibility to B. pahangi infection.

Previous reports characterizing the Brugia pahangi-jird model have demonstrated that the preferred developmental site within male jirds is the testes, and the spermatic cord duct and iliolumbar lymphatics (Ash and Riley, 1970; Ash, 1971; Ah and Thompson, 1973). The altered location of adult worms reported in the current study may be due to the testicular and lymphatic immaturity of 2-wk old jirds.

Pathologic responsiveness, expressed as the mean number of intralymphatic thrombi produced per intralymphatic worm, did not significantly differ between the 4 age groups of infected jirds. Our observation that jirds infected at 2 wk of age produce fewer intralymphatic thrombi than the other 3 age groups of infected jirds is not surprising, and is undoubtedly due to fewer adult B. pahangi present in the testes and associated lymphatic vessels of these younger male jirds.

Reduced IgG antibody responses of jirds following B. pahangi infection at 2 wk of age may be due to the reduced

numbers of circulating lymphocytes observed in younger jirds in this and previous studies (Termer and Glomski, 1978). Immature T helper cells incapable of initiating isotype switching, as described in young mice (Mosier and Johnson, 1975), may also contribute to lower levels of antiparasite IgG antibodies in the sera of jirds infected at 2 wk of age. Interestingly, the significantly reduced antiparasite antibody responses of jirds infected when 2 wk old had no effect on either susceptibility or intralymphatic pathologic responsiveness to B. pahangi.

No differences were measured in susceptibility or pathologic responsiveness of male jirds to experimental infections with B. pahangi as a function of either age, relative levels of testosterone, or antibody responsiveness. Infections initiated in 2-wk old male jirds did result in reduced numbers of intralymphatic thrombi as a consequence of predominantly extralymphatic adult worm location. This altered location of adult worms may simply reflect an unsuitable testicular and lymphatic environment for worm development in jirds infected at 2 wk of age. Although direct comparisons between jirds and humans are difficult, the possibility of extralymphatic adult parasite development and its potential effect(s) on immune responses and lymphatic pathologic changes during human lymphatic filariasis in children or adults, remains unclear.

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Table I. Summary of parasitological data from necropsies performed 60 days after s.c. inoculation of 100 *B. pahangi* L_3 into 2-, 6-, 10-, and 15-wk old male jirds.

			Adult Worm Recovery*			
Group	n	Patency	Lymphatic	Heart/Lung	Peritoneal	Total
2wk	9	0%	5.3 ± 4.7[a]	11.1 ± 8.9	7.7 ± 7.3	24.1 ± 19.0
			(22.0%)	(46.1%)	(31.9%)	
6wk	10	20%	23.4 ± 8.2[b]	8.3 ± 8.7	1.0 ± 1.0	32.7 ± 13.5
			(71.6%)	(25.4%)	(3.0%)	
10wk	9	22%	31.8 ± 16.4[b]	3.4 ± 4.9	1.2 ± 1.8	36.4 ± 13.9
			(87.4%)	(9.3%)	(3.3%)	
15wk	10	10%	30.6 ± 10.9[b]	2.9 ± 7.1	1.8 ± 1.8	35.3 ± 9.8
			(86.7%)	(8.2%)	(5.1%)	

*Expressed as the mean adult worm recovery ± SD, and the percent of the total mean worm recovery in parentheses below. Letters, where present, indicate significant differences ($p \leq 0.05$) between mean ranks within columns as measured by the nonparametric equivalent of the Student-Newman-Keuls test.

Table II. Serum antibody responses of jirds infected at 2, 6, 10, and 15 wk of age to soluble adult *B. pahangi* antigens.

		Serum IgG Antibody				
Group	n	Wk0*	Wk2	Wk4	Wk6	Wk8
2wk	7	0.0	0.0[a]	1.6 ± 2.0[a]	9.4 ± 4.6[a]	25.2 ± 19.0[a]
6wk	9	0.0	0.9 ± 1.0[a]	33.7 ± 18.1[b]	41.0 ± 15.8[b]	97.7 ± 36.4[c]
10wk	9	0.0	0.8 ± 0.6[a]	29.6 ± 17.1[b]	30.0 ± 6.1[b]	80.0 ± 35.8[bc]
15wk	10	0.0	2.9 ± 2.4[b]	27.1 ± 16.8[b]	31.4 ± 15.2[b]	52.1 ± 30.4[ab]

*Serum antibody levels measured at 0, 2, 4, 6, and 8 wk PI are reported in ELISA units as the mean ± SD of values judged from a standard curve included with each assay. Letters indicate significant differences ($p \leq 0.05$) between group means within columns as determined by the Tukey test.

CHAPTER 3

Brugia pahangi: Reduced Antibody Responsiveness During Homologous Challenge Infection in Male Progeny of Infected Female Jirds

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To be submitted to Experimental Parasitology

ABSTRACT - Alteration of the immune and inflammatory responses of offspring to homologous infections due to *in utero* and neonatal exposure to maternal *B. pahangi* infections was studied in age-matched male progeny of infected and uninfected female jirds. Infections initiated in 2-wk old offspring yielded overall $\bar{X}$ ± sd adult worm recoveries of 6.0 ± 5.7 and 4.2 ± 5.4 in offspring from infected or uninfected mothers, respectively. Infections initiated in 4-wk old offspring resulted in an overall $\bar{X}$ ± sd recovery of adult worms of 11.3 ± 11.3 and 10.2 ± 5.8 in offspring from infected and uninfected mothers, respectively. The ratio of intralymphatic thrombi per intralymphatic worm varied between experiments, but did not significantly differ within experiments between infected offspring from infected or uninfected mothers. Areas of granulomatous inflammatory responses measured around *B. pahangi* antigen-coated beads embolized in the lungs was not significantly affected by maternal origin in either age group of infected or uninfected progeny. Granuloma areas around *B. pahangi* antigen-coated beads in the lungs of offspring infected at 2 wk of age from infected females, however, were consistently smaller than age-matched infected offspring from uninfected females. Offspring infected at either 2 or 4 wk of age from infected female jirds exhibited significantly reduced titers of serum IgG antibodies to *Brugia* antigens compared to infected offspring of uninfected

mothers at 5-8 wk postinfection. Infected offspring from infected females also had significantly fewer splenic plaque-forming cells to B. pahangi antigens at 5 wk postinfection than similarly infected offspring from uninfected females. Western immunoblot analysis indicated both qualitative and quantitative reductions in serum antibody reactivity to adult B. pahangi antigens in infected progeny of infected females of both ages compared to age-matched infected controls. Reduced homologous serum antibody responses in progeny exposed to maternal B. pahangi infections suggests a partial immune tolerance to filarial antigens. The reduced antibody responsiveness to B. pahangi antigens observed in the infected offspring from infected females had no demonstrable effect, however, on adult worm burdens, microfilaremias, the development of lymphatic lesions, or antigen-specific granulomatous inflammatory responses in comparison to the infected progeny of uninfected females.

Index Descriptors and Abbreviations: Nematoda, Filarioidea, *Brugia pahangi*, filariasis, neonatal tolerance, maternally-acquired immunity, jird, *Meriones unguiculatus*; Immunoglobulin G [IgG], Enzyme-linked immunosorbent assay [ELISA], Third stage infective larvae [L_3], Soluble adult *Brugia pahangi* antigenic preparation [BpAg], Diethanolamine [DEA], Cyanogen bromide-activated Sepharose 4B [CNBS], 0.01M Phosphate buffered saline [PBS], 0.1% Tween 20 in PBS [PBST], Fetal calf serum [FCS], Horseradish peroxidase [HRP], Immunoglobulin [Ig], Optical density measured at 630nm [OD_{630}], Optical density at 490nm [OD_{490}].

INTRODUCTION

Human lymphatic filariasis is a complex disease consisting of a variety of pathological changes in infected individuals. Some, but not all, of the host's pathologic changes are thought to be immunologically mediated (Otteson, 1980). The establishment of "categories" of clinical disease states encountered within infected populations has aided the understanding of the host's immune responses and their association with the pathogenesis of lymphatic filariasis (Otteson, 1980; 1984; 1989). Reasons for the variability in immunologic reactions and clinical states of disease observed in infected people are still unclear. Interestingly, transmigrants into areas endemic for filariasis demonstrate stronger immune responses to filarial antigens, an accelerated course of clinical disease, and a lower incidence of microfilaremias following infections compared to the indigenous population (Partono, 1987). One hypothetical explanation for these variable immune responses and clinical diversity in the lymphatic filariases is that <u>in utero</u> and/or neonatal exposure to maternal filarial infections may alter the immune responses of the progeny, and thus may alter expression of clinical disease following subsequent homologous infection (Otteson, 1980; Otteson, 1984; Piessens et al., 1987). Evidence that fetal exposure to filariae, filarial antigens, or maternal antifilarial

immunity can affect the responses of the progeny comes from studies of both humans and experimental models. Antifilarial antibodies of the IgM (Dissanayake et al., 1980) and IgE isotypes (Weil et al., 1983) have been demonstrated in cord blood of babies born to Wuchereria bancrofti-infected mothers. These antibody isotypes do not readily cross the human placenta, and were suggested to be produced by the fetus in response to in utero sensitization to filarial antigens. Transplacental transfer of Acanthocheilonema viteae microfilariae in rats has been shown to abrogate the normal expression of homologous protective immunity against challenge infection in the progeny of infected mothers (Haque and Capron, 1982). Schrater et al, (1983) observed an increased incidence of microfilaremias in infected female offspring of B. malayi-infected mothers compared to infected female progeny of uninfected female jirds. Similar observations on the increased expression of microfilaremias in the infected male progeny of infected mothers were made in the jird-B. pahangi model (Klei et al., 1986). Infected male offspring from B. pahangi-infected female jirds also displayed reduced lymphatic lesion severity compared to the infected male offspring of uninfected females (Klei et al., 1986).

The severity of lymphatic lesions in B. pahangi-infected jirds can be either increased by immunization with homologous antigens (Klei et al., 1982), or decreased during

chronic infections (Klei et al., 1988; 1990). Reductions in pulmonary granulomatous inflammatory response areas around B. pahangi antigen-coated beads coincide with the reduced lymphatic lesion severity observed in chronic infections (Klei et al., 1990). Formation of both intralymphatic lesions and pulmonary granulomas in infected jirds appear to have a similar immunological component which may be altered in offspring exposed to maternal B. pahangi infections. The purpose of the present paper is to use this artificial lung granuloma assay to confirm and extend previous observations of reduced lymphatic lesion severity in the male progeny of infected female jirds (Klei et al., 1986), and to more critically evaluate the immunologic and pathologic reactions of male progeny of B. pahangi-infected and uninfected female jirds to homologous challenge infections.

MATERIALS AND METHODS

Brugia pahangi was maintained in jirds and Aedes aegypti mosquitos. Infective L_3 were obtained by crushing cold-anesthetized mosquitos 11 days after ingestion of infected jird blood by previously described techniques (Bosshardt et al., submitted). Doses of active L_3 were counted with the aid of a stereo microscope and inoculated in ≤ 1.0ml of RPMI 1640 supplemented with 25mM Hepes buffer and 2% antibiotic/antimycotic solution (Grand Island Biological Co., Grand Island, NY).

Male and female jirds (Meriones unguiculatus) used as monogamous breeding pairs were obtained commercially (Tumblebrook Farms, West Brookfield, MA) and maintained on Purina lab chow and water ad lib. Offspring from infected mothers were derived from female jirds inoculated IP ≥ 100 days previously with 100 B. pahangi L_3. A minimum of 24 male offspring, 12 from each maternal source, comprised an experimental group. Half of the male progeny (n ≥ 6) from both infected (Group I) and uninfected (Group U) female jirds were inoculated SC in the left inguinal region with 100 B. pahangi L_3 at either 2 or 4 wk of age. The other half of the male progeny served as uninfected controls. All offspring were bled weekly via the retro-orbital plexus to collect serum. All progeny within a given experiment were necropsied on the same day. Necropsies were performed at

times ranging from 63 to 75 days postinfection. Adult worm burdens and lymphatic lesion severity were measured at necropsy by previously described methods (Klei et al., 1982). Microfilaremias were determined at necropsy by the modified Knott's test (Knott, 1939) using 0.5 ml of whole blood; or by microscopic examination of 20 ul of heparinized whole blood.

Measurements of granulomatous inflammatory response areas around BpAg-CNBS embolized in the lungs of infected and uninfected male progeny from both maternal sources were accomplished at necropsy as previously described (Klei et al., 1988). Briefly, CNBS were coupled with either BpAg, prepared as previously described (Klei et al., 1982), or DEA by previously described techniques (Axen et al., 1967). Pulmonary emboli were produced in jirds 3 days prior to necropsy by retro-orbital inoculation of 2.5×10^4 coupled CNBS per animal. Lungs were perfused via the trachea with 10% buffered formalin at necropsy, embedded in glycol methacrylate (Polysciences, Warrington, PA), and 2.5 um step sections cut at 50 um intervals. Granulomas were traced from lung cross sections with the aid of a compound microscope with an attached drawing tube at a magnification of 400x. Tracings of 25 inflammatory foci per animal were measured using a digitizing pad linked to an IBM-PC equipped with Bioquant IV software (R & M Biometrics, Inc., Nashville, TN). Granuloma areas are expressed in um^2.

Lymphocyte blast transformation assays were performed at necropsy by previously described methods (Prier and Lammie, 1988). Lymphocytes obtained from the spleen and lymph nodes of infected and uninfected progeny were stimulated in vitro with a 1:500 dilution of phytohemagglutinin (Sigma Chemical Co., St. Louis, MO), a 1:100 dilution of pokeweed mitogen (Grand Island Biological Co., Grand Island, N.Y.), or 20 ug/ml BpAg in RPMI 1640 supplemented with Hepes buffer (25 mM), L-Glutamine (2mM), penicillin/streptomycin/amphotericin B solution (1%), and 2% fetal calf serum (Grand Island Biological Co., Grand Island, N.Y.). Mitogen-stimulated cells were incubated for 3 days in flat-bottomed plates, and antigen-stimulated cells were incubated for 5 days in round-bottomed plates, at 37°C in a humidified atmosphere of 5% CO_2 in air. Incorporation of ^{3}H-thymidine was measured after a 24 hour pulse period. Autologous unstimulated cells were used for the calculation of the stimulation indices obtained by dividing the counts per minute of stimulated cultures by the counts obtained from autologous unstimulated cultures.

Serum IgG antibodies to BpAg in infected and uninfected male progeny of Group I and Group U mothers were quantitated by ELISA procedures. Immulon I flat-bottomed, 96-well polystyrene microtiter plates (Dynatech Laboratories, Inc., Alexandria, VA) were coated overnight with 50 ul/well of a 15 ug/ml solution of BpAg in 0.02M carbonate buffer, pH 9.6.

Plates were then washed 3X for 5 min each with 0.2 ml/well PBST before serial two-fold dilutions of sera were performed in each plate. Alternately, samples of test serum were diluted 1:100 in PBST and added in duplicate at 50 ul/well. Plates were washed after a 20 min incubation at 20°C before addition of 50 ul/well of a 1:2000 dilution in PBST of rabbit antiserum raised against jird immunoglobulins purified by Protein A chromatography techniques previously described (Miller and Stone, 1978). Plates were incubated for 45 min at 20°C and washed. A 1:1000 dilution of antirabbit IgG conjugated with HRP (Kirkegaard and Perry Laboratories, Inc., Gaithersburg, MD) in PBST was added at 50 ul/well, incubated an additional 45 min at 20°C, and washed as before. Substrate solution consisting of 0.005M 5-aminosalicylic acid, pH 5.7, and 0.0005% H_2O_2 was added at 100 ul/well, and the reaction stopped with 1N NaOH after 20 min in the dark at 20°C. Titers of anti-BpAg antibodies measured in ELISA were determined as the $\log_2$ dilution of whole test serum yielding an OD_{630} of $\geq$ 2X the OD_{630} obtained using a pool of uninfected jird sera; or as ELISA units determined by plotting the average OD_{630} of duplicate wells against a standard curve generated with a known positive serum pool included in each plate.

Serum antibodies to BpAg in infected jirds were qualitatively assessed by Western immunoblot. Parasite antigens were separated by sodium dodecyl sulfate

polyacrylamide gel electrophoresis using a 7.5-20% acrylamide gradient separating gel and methods previously described (Laemmli, 1970). Separated BpAg and prestained molecular weight markers (Bethesda Research Laboratories, Bethesda, MD) were transblotted from the gel to 0.22um nitrocellulose paper (Schleicher and Schuell, Inc., Keene, NH), and 3mm strips removed for immunoblotting after staining with 0.5% Ponceau S dye. Nitrocellulose paper strips were blocked for 30 minutes at 20°C with a solution of 5% w/v nonfat dry milk and 0.3% Tween 20 in PBS. Normal and test sera were diluted 1:100 in PBST and incubated with individual strips for 90 minutes at 20°C. Rabbit antijird IgG and goat antirabbit Ig conjugated with HRP (Pel-Freez Biologicals, Rogers, AR) were diluted separately in PBST at 1:2000 and 1:1000, respectively, and sequentially incubated with the strips for 90 minutes each at 20°C. Strips were washed 3X for 10 minutes each between antibody incubation steps, and prior to the addition of substrate solution consisting of 35mg 4-chloro-1-naphthol in 50ml of 0.05M Tris-HCl, pH 6.8, plus 10ml absolute methanol and 30ul of a 30% H_2O_2 solution. The substrate reaction was stopped with distilled water.

Densitometric analysis of Western blot strips reacted with pooled sera from infected and uninfected progeny from both maternal sources was performed with an Ephortec™ densitometer (Joyce-Loebl, Tyne & Wear, U.K.). Immunoblot

strips were immersed together in antibody and substrate solutions subsequent to the initial reactions with test sera for uniform development. Densitometry was performed on individual strips within 3 days of immunoblotting using a 0.1mm aperture width. Densitometric profiles are expressed as the absorbance of reflected light at 626nm.

Splenocytes producing IgG antibodies to BpAg in infected and uninfected progeny of both maternal sources were enumerated by previously described methods (Wassom et al., 1986). Filter immunoplaque assays were modified for a Dotblot apparatus (Biorad Laboratories, LaJolla, CA) by incubating 3.0×10^5 cells/well on BpAg-coated nitrocellulose paper for 2.5 hr under the previously described cell culture conditions. Antibody-producing cells were then detected using the same rabbit antijird IgG, HRP-conjugated goat antirabbit antibodies, and substrate used in Western immunoblots. Plaques were counted with the aid of a 30X stereo microscope.

Levels of a B. pahangi antigen containing a phosphorylcholine epitope were measured in the serum of mothers from whom experimental progeny were obtained as described (Weil et al., 1988). A 20 ug/ml solution of a murine monoclonal antibody recognizing a B. malayi antigen containing phosphorylcholine in 0.1M bicarbonate buffer, pH 8.0, was added at 100 ul/well to 96-well, polyvinyl, round bottomed microtiter plates (Dynatech Laboratories, Inc.,

Alexandria, VA) and incubated overnight at 37°C. Plates were washed 3X with PBST before the addition of each new reagent. Coated plates were blocked with 200 ul/well PBST containing 5% FCS for 30 min. Maternal sera were pretreated by heating in a 100°C water bath for 5 min in the presence of EDTA solution, pH 7.5, and PBS to remove interfering material prior to use in the assay. Serial two-fold dilutions of 100 ul/well of pretreated sera were performed in each plate at a beginning dilution of 1:10 in PBST containing 5% FCS. The same monoclonal antibody recognizing phosphorylcholine conjugated to HRP and diluted 1:2000 in PBST plus 5% FCS was then added at 100 ul/well and incubated for 1-2 hours. Substrate solution consisting of o-phenylenediamine and H_2O_2 was added at 100 ul/well and the reaction stopped after 10 min at 20°C with 4M H_2SO_4. Optical densities were measured against a PBST blank at 490nm. Maternal serum antigenemias are expressed as the serum dilution which gave an $OD_{490} \geq$ the $\bar{X} \pm 3SD$ of the OD_{490} obtained from normal sera included in each plate.

Granuloma areas were compared by analysis of variance. Significant differences between group means were assessed by the Tukey test. Serum IgG antibody titers and numbers of splenic plaque-forming cells from infected and uninfected progeny were compared by the Student's t-test. Worm recoveries and numbers of intralymphatic thrombi from infected progeny were compared by the Mann-Whitney U-test.

Lymphocyte blastogenesis data were analyzed by the nonparametric Kruskal-Wallis test, and significant differences between group mean ranks were judged by the nonparametric equivalent of the Student-Newman-Keuls test (Zar, 1984). Arithmetic and geometric group means are reported ± SD. Differences between group means were considered significant at $p \leq 0.05$.

RESULTS

Male progeny used in challenge infection experiments were obtained from 15 B. pahangi-infected and 15 uninfected female jirds. Group I and Group U mothers came from two separate sets of infected and uninfected female jirds that were necropsied at either 11 or 14 months postinfection. Parasitological and immunological data obtained from necropsies of Group I mothers providing offspring is summarized in Table I. Adult B. pahangi were typically found loose in the peritoneal cavities of Group I mothers. Lymphatic-dwelling worms were detectable in only 3/15 Group I mothers, yet 67% (10/15) expressed microfilariae in their blood at necropsy. Group I mothers with 0 detectable adult worms at necropsy (n=4) displayed either circulating or splenic microfilariae. One Group I mother which had no measurable PC-containing antigen in circulation had a single adult worm in the heart at necropsy. Group I mothers, not surprisingly, demonstrated significantly stronger responses to BpAg than Group U mothers as measured by serum IgG antibody titers and granulomatous reactions in the lungs. Group U mothers displayed $\overline{X} \pm$ sd pulmonary granuloma areas of $5175 \pm 1021um^2$ around BpAg-CNBS, and $4351 \pm 715um^2$ around DEA-CNBS. Group U mothers were initially negative for serum antibodies to BpAg, but developed background levels of 1-2 by necropsy.

Parasitological data from five experiments in which offspring from both maternal sources were infected at either 2 or 4 wk of age are summarized in Table II. Adult B. pahangi recoveries and anatomical distribution were similar within experiments in offspring groups infected at 2 or 4 wk of age from either Group I or Group U mothers. The percentage of patent infections and levels of circulating microfilariae were also similar between offspring groups infected at 2 or 4 wk of age from both maternal sources.

Offspring from Group I and Group U females had equivalent numbers of intralymphatic thrombi within offspring groups infected at 2 or 4 wk of age (Table III). Maternal source had no measurable effect on distribution of intralymphatic thrombi between spermatic cord duct and iliolumbar lymphatic vessels in infected age-matched progeny. The degree of lymphatic inflammatory reactivity, expressed as the mean number of intralymphatic thrombi formed per intralymphatic worm recovered, was also equivalent between offspring groups within each experiment. Jirds infected at 2 wk of age had fewer adult worms and lymphatic thrombi than jirds infected at 4 wk of age. Direct comparisons between these age groups, however, cannot be made due to infections initiated with different batches of B. pahangi L_3.

Inflammatory responses measured in the lungs of infected and uninfected offspring around embolized BpAg-CNBS

indicated consistent, albeit insignificant, reductions in granuloma areas in the lungs of offspring infected at 2 wk of age from Group I mothers compared to similarly infected progeny of Group U mothers in 2 experiments (Table III). Maternal source had no significant effect on pulmonary inflammatory responses to BpAg-CNBS in offspring infected at 4 wk of age. Mean areas of granulomatous reactions in the lungs of uninfected progeny from both maternal groups to BpAg- or DEA-CNBS were similar (data not shown).

Stimulation indices of spleen cells from infected offspring stimulated in vitro with BpAg were expectedly higher than those of uninfected progeny, but no significant differences in proliferative responses to mitogens or BpAg were measured between infected Group I and Group U offspring (data not shown). The overall $\overline{X} \pm$ sd stimulation index to BpAg obtained with splenocytes of offspring infected at 4 wk of age was 14.4 ± 10.2 and 25.4 ± 35.3 in progeny from Group I and Group U mothers, respectively. The splenocyte blastogenic reactivity to BpAg of uninfected offspring from both maternal sources for these experiments was similar with group $\overline{X}$ stimulation indices ranging from 0.7-2.7. The overall $\overline{X} \pm$ sd stimulation index to BpAg obtained with lymph node cells of infected progeny were 7.1 ± 5.4 and 6.1 ± 6.4 for offspring from Group I and Group U mothers, respectively. Lymph node cell responses to BpAg of all uninfected progeny in experiments initiated at 4 wk of age

yielded $\overline{X}$ stimulation indices of approximately 1. The *in vitro* blastogenic responses of spleen and lymph node cells from progeny in experiments initiated in 2 wk old progeny were similar to those in experiments begun in 4 wk old offspring.

Representative serum antibody levels to BpAg of progeny infected at 4 wk of age from Group I and Group U mothers are shown in Figure 1. Initial serum IgG antibody levels to BpAg in the progeny of Group I mothers were always high, but waned with time in uninfected offspring. Infected Group I progeny demonstrated little increase in serum antibody levels to BpAg during the first 3 wk of infection. Infected offspring of Group U mothers, on the other hand, displayed sharply increasing serum antibody levels to BpAg by 3 wk postinfection. The serum IgG antibody titers of infected Group U progeny to BpAg increased to significantly higher levels than infected Group I progeny by 5 wk postinfection. Significant differences between serum IgG antibody titers to BpAg of Group I and Group U progeny was seen in all experiments between 5 and 8 wk postinfection.

Filter immunoplaque assays were performed at 5 wk postinfection with offspring from both maternal sources infected at 4 wk of age to measure numbers of splenic IgG plaque-forming cells to BpAg (Table IV). Infected offspring from Group U mothers displayed significantly higher mean numbers of splenic plaque-forming cells to BpAg than

infected progeny of Group I mothers at 5 wk postinfection. Numbers of background plaques from uninfected progeny of both maternal groups were negligible. Levels of serum antibodies to BpAg in the animals used for these assays reflected the numbers of splenic plaque-forming cells to BpAg.

Western immunoblot analyses of serum IgG antibody recognition of BpAg by offspring infected at 2 or 4 wk of age from both maternal sources is shown in Figure 2. Pooled serum collected at a time of maximum antibody levels (8 wk postinfection) showed that serum antibodies from infected progeny of Group I females reacted with fewer parasite antigens than sera from Group U offspring. This lack of antibody recognition by infected Group I progeny was especially apparent against antigens with M_r of $\leq$ 60 kd. Densitometer readings of these immunoblot results (Figure 3) further indicated that serum IgG antibodies from infected Group I progeny reacted to BpAg more weakly than antibodies from infected Group U progeny. The reduced serum antibody reactivity observed in infected Group I progeny compared to infected Group U progeny occurred to antigens throughout the range of molecular weights recognized by antibodies from the sera of both infected offspring groups.

DISCUSSION

The offspring of B. pahangi-infected mothers reported here consistently displayed lower serum titers of IgG antibodies to BpAg than offspring from uninfected mothers at 4-8 wk postinfection. Immunofilter plaque assays conducted at 5 wk postinfection in uninfected and infected progeny from each maternal group demonstrated that infected offspring of infected mothers had fewer splenic antibody-producing cells to BpAg than infected progeny of uninfected mothers. Experiments with other rodents have indicated that progeny of a mother immunized against erythrocyte antigens (Yamaguchi et al., 1983; Izuchi et al., 1985; Sharmanov et al., 1986; Koshimo et al., 1989), or ovalbumin (Koshimo et al., 1989;) produce fewer plaque-forming cells compared to control offspring after immunization of both groups of progeny with the homologous antigen. Potential mechanisms for the observed reduction in antibody responsiveness to BpAg in infected jirds exposed to maternal filarial infections include the induction of suppressor cell networks in the progeny (Okamoto et al., 1989), the transplacental transmission of antigens (Gill et al., 1973), antigen-antibody complexes transferred to the progeny in the milk (Auerbach and Clark, 1975), or the presence of immunomodulatory maternal antibodies (Yamaguchi et al., 1983; Izuchi et al., 1985; Sharmanov et al., 1986; Okamoto

et al., 1989) leading to a state of neonatal immunologic tolerance or suppression. The most obvious explanation for the reduced serum antibody titers and plaque-forming cells to BpAg in the infected progeny of infected females is the presence of high titers of serum IgG antibodies of maternal origin previously described in the uninfected progeny of infected mothers (Bosshardt et al., submitted). The exact mechanism(s) for this reduced antibody responsiveness observed in infected Group I progeny, however, remains uncertain.

Infected male offspring from B.pahangi-infected female jirds showed no differences in susceptibility to homologous challenge infection when compared to the offspring of uninfected mothers in this and previous studies (Klei et al., 1986). Both infected and uninfected progeny of Group I female jirds also showed no effects of exposure to maternal filarial infections on antigen-stimulated blastogenic responses of their spleen and lymph node cells when compared to similarly infected or uninfected Group U progeny. The progeny of Acanthocheilonema viteae-infected female rats have demonstrated susceptibility to homologous challenge infections unlike the normally refractory progeny of uninfected female rats (Haque and Capron, 1982). This unusual susceptibility to A. viteae infections in progeny of homologously infected mothers occurred while microfilariae transferred in utero were still present in the circulation

of the uninfected progeny; and at a time when their splenocyte blastogenic responses demonstrated an antigen-specific tolerance to A. viteae antigens. Similar studies in mice have shown that the progeny of A. viteae-infected females remained refractory to A. viteae infections like the progeny of uninfected mothers (Storey et al., 1988). Splenocyte blastogenic responses in these uninfected murine progeny of infected mothers were enhanced to A. viteae antigens compared to the progeny of uninfected mothers even in the presence of microfilaremias derived in utero (Storey et al., 1988). Microfilariae of both Wuchereria bancrofti (Bloomfield et al., 1978; Rao et al., 1984) and Onchocerca volvulus (Brinkmann et al., 1976) can cross the human placenta potentially influencing the development of antifilarial immune responses in the progeny. Additionally, cordblood IgM (Dissanayake et al., 1980) and IgE (Weil et al., 1983) antifilarial antibodies detected in babies born to W. bancrofti-infected mothers suggest in utero sensitization to filarial antigens can occur. Microfilariae of B. pahangi have not, however, been reported to cross the jird placenta (Bosshardt and Klei, unpublished observations), although they have been documented to be transmitted in utero in rats (Sucharit and Rongsriyam, 1980) and cats (Kimmig, 1979). A 105-110 kd molecular weight B. pahangi antigen was not detectable in neonatal jird sera at a time when it was demonstrable in the sera of their

infected mother (Weil et al., 1990). These results taken together suggest that transplacental transfer of microfilariae, or filarial antigens, leading to alteration of homologous *in vitro* blastogenic responses and/or protective immune responses to homologous infections in the progeny of filariae-infected mothers may be a host-specific event which does not occur in the jird-*B. pahangi* model.

Transmigrants into areas endemic for lymphatic filariasis rarely exhibit patent infections (Wartman, 1947). This is not the situation in people born to mothers living within areas endemic for filariasis who apparently respond poorly to filarial infections and freely express microfilaremias (Partono, 1987). Homologously infected offspring of both *B. pahangi*- (Klei et al., 1986) and *B. malayi*-infected female jirds (Schrater et al., 1983) have demonstrated a higher percentage of patent infections than infected offspring from uninfected mothers. No significant differences in percent patency or levels of microfilaremias were observed between infected offspring from Group I or Group U female jirds in the current experiments. Infections reported here were terminated near the onset of *B. pahangi* patency in infected jirds (Ash, 1971) in order to measure maximum lymphatic lesions occurring during the acute phase of reactivity to *B. pahangi* in jirds (Klei et al., 1990). Differences in duration of *B. pahangi* infections in this and the previous study (Klei et al., 1986) could account for the

current lack of evidence for a predisposition of infected Group I offspring for the expression of microfilaremias compared to infected Group U offspring.

Intralymphatic lesion formation measured in infected progeny from both maternal sources did not indicate any differences in either total numbers of intralymphatic lesions formed, or the ratio of number of intralymphatic thrombi per intralymphatic worm, in the infected offspring of infected or uninfected mothers. Previous studies indicated that male progeny of homologously infected female jirds exhibited reduced lymphatic lesion severity compared to similarly infected progeny of uninfected female jirds (Klei et al., 1986). The kinetics of lymphatic lesion formation of jirds infected with B. pahangi (Klei et al., 1990) indicates, however, that the lymphatic lesions measured here between 65 and 75 days postinfection occurred during a more acute phase of maximum pathological responsiveness to the parasite than the 110-120 day infections reported previously (Klei et al., 1986). Pathologic reactivity to the parasite may not be directly comparable at these two time points. Necropsies were performed at a time of maximum lymphatic lesion formation because any maternally-acquired down regulation of Group I offspring responses might lead to maximal differences in lesion severity between infected offspring groups at this time. An alternate explanation for the lack of reduced

lymphatic lesion formation in Group I progeny in the current study is that any immunologic down regulation induced in progeny of infected mothers may not overcome other nonimmune factors contributing to lymphatic lesion formation during this acute phase. Furthermore, Group I mothers in this report were inoculated with fewer B. pahangi L_3 than mothers used in the previous study (Klei et al., 1986) in order to increase the numbers of experimental male offspring produced. Both previous studies (Klei et al., 1986) and attempts to use females inoculated with 200 B. pahangi L_3 IP as breeders in studies subsequent to the current report have proven that heavily infected female jirds produce very few offspring (Bosshardt and Klei, unpublished observations). Reduced intensity of maternal infection used here, however, could have reduced the effects of in utero and neonatal exposure to B. pahangi which led to the altered responses of the progeny observed previously in this model (Klei et al.,1986). The results of the current report do not, therefore, necessarily contradict these previous findings of reduced lymphatic lesion severity in the infected male progeny of infected female jirds.

Progeny infected at 2 weeks of age, but not 4 weeks of age, from infected mothers demonstrated consistently, albeit insignificantly, smaller pulmonary granulomas to BpAg-CNBS than infected control offspring. Lewert and Mandlowitz (1969) demonstrated that granulomatous hypersensitivity

reactions around S. mansoni eggs embolized in the lungs of uninfected mice born to homologously infected females were smaller than in the offspring of uninfected mice. Rodent maternal antibodies transferred to their progeny have shown the ability to modulate the neonate's T-cell responsiveness through anti-idiotypic interactions (Martinez et al., 1986). Uninfected progeny of B. pahangi-infected female jirds have demonstrated peak titers of maternally-acquired anti-BpAg serum antibodies at approximately 2 wk of age which are waning by 4 wk of age (Bosshardt et al., submitted). These maternal antibodies to BpAg in neonatal circulation were shown to have no measurable effect on the formation of pulmonary granulomas to embolized BpAg-CNBS in the uninfected 4- or 8-wk old progeny from Group I mothers compared to the progeny of uninfected females. The lack of measurable effect of these maternal antibodies to BpAg on the homologous pulmonary inflammatory responses of their progeny was thought to be due to either an absence of the appropriate antigens coating the embolized beads, or the inability of maternal antibody to induce down-regulated granulomatous inflammatory responses in these uninfected progeny. Measurements of cellular responses around BpAg-CNBS embolized in the lungs provide a more sensitive indicator of the granulomatous inflammatory reactivity of infected jirds to B. pahangi antigens than counting intralymphatic thrombi numbers or determining lymph vessel

dilation. Results of the current study, however, indicate that the presence of high serum titers of maternal antibodies to BpAg in 2-wk old, but not 4-wk old, Group I progeny infected with B. pahangi may induce partial down-regulation of homologous pulmonary granulomatous reactivity compared to the infected progeny of uninfected mothers.

The results of these experiments taken together suggest that maternal filarial infections and/or their associated antifilarial immune effectors have a tolerizing effect on the antibody responses of their progeny during homologous infections. Maternal effects on the homologous cellular inflammatory responses to infection are not as easily demonstrable. The complex effects that exposure to maternal filariasis may have on the subsequent cellular and antibody responses of their progeny to homologous antigens remain to be further elucidated.

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Table I. Summary of parasitological and immunological data from 15 infected female jirds that produced male offspring for subsequent experiments.

B. pahangi Recovery			ELISA Antibody**		Pulmonary Granulomas**	
Adult	Mf	PC-Ag	Initial	Necropsy	DEA	Brugia
4.7 ± 5.7*	31.3 ± 54.9*	1:82†	10.9 ± 1.1	10.0 ± 1.3	4624 ± 627	7551 ± 1721
(0-19)	(0-166)	(0-1:320)	(9-12)	(7-12)	(3806-5409)	(5257-10,066)

Footnotes for Table I.

* $\bar{X}$ ± sd recovery of adult parasites, or microfilariae per 20 ul of whole blood, determined at necropsy. The range of parasite recoveries is in parentheses below.

+ $\bar{X}$ titer, and range of titers in parentheses below, of circulating phosphorycholine-containing B. pahangi antigen measured in maternal sera at or near the time of birth of experimental male progeny. Antigenemia titers were judged as the serum dilution yielding an OD_{490} which was ≥ the $\bar{X}$ ± 3sd OD_{490} obtained from normal sera included in each plate.

** Geometric $\bar{X}$ ± sd $\log_2$ titer measured at 100 days postinfection (initial) and at necropsy judged as the serum dilution which gave an OD_{630} ≥ 2x the OD_{630} obtained from pooled uninfected sera included in each plate.

++ $\bar{X}$ ± sd granuloma areas around DEA-CNBS or BpAg-CNBS are expressed in um^2, with the range of granuloma areas represented in parentheses below..

Table II. Summary of parasitological data from necropsies following homologous challenge infections of 2 or 4 wk old male progeny from B. pahangi-infected and uninfected mothers.

					B. pahangi Recovery					
					Adult Worms[++]			Microfilariae[++]		
Age	Exp	Mother	n	PI	Lymph	Heart	Peritoneal	Total	mf	Patent
2[*]	1	U	6[+]	67[**]	0.8 ± 1.3	0.2 ± 0.4	0.7 ± 1.0	1.7 ± 2.7	1.8 ± 4.5	17%
		I	7	67	2.6 ± 2.6	0.1 ± 0.4	0.3 ± 0.5	3.0 ± 2.4	11.0 ± 18.2	43%
2	2	U	6	67	2.3 ± 3.4	1.0 ± 2.0	1.8 ± 2.6	6.7 ± 6.4	0.5 ± 1.2	17%
		I	4	67	4.5 ± 5.8	3.5 ± 4.7	3.3 ± 2.8	11.3 ± 6.4	1.3 ± 1.9	50%
4	1	U	5	60	17.0 ± 12.8	1.7 ± 2.3	1.5 ± 1.4	20.2 ± 12.5	24.2 ± 22.9	80%
		I	6	60	13.0 ± 10.3	3.8 ± 6.6	0.7 ± 1.0	17.5 ± 9.5	2.2 ± 4.4	33%
4	2	U	6	62	13.9 ± 11.3	0.8 ± 1.9	0.4 ± 1.1	15.1 ± 11.5	0.0	0%
		I	7	62	8.3 ± 6.8	1.5 ± 2.7	0.0	9.8 ± 8.8	0.0	0
4	3	U	6	73	8.0 ± 5.6	1.5 ± 1.5	0.7 ± 1.0	10.2 ± 5.8	2.3 ± 2.0	67%
		I	6	73	10.3 ± 11.0	0.0	1.0 ± 2.0	11.3 ± 11.3	1.8 ± 2.0	50

Footnotes for Table II.

* Offspring age in weeks at time of challenge infection.

+ Unequal n resulted from the deaths of jirds after infection.

** Duration of homologous challenge infections in days at necropsy.

++ Adult worm recoveries and levels of microfilariae in circulation are expressed as the $\bar{X}$ ± sd. Microfilariae levels were measured in 0.5 ml of whole blood by the modified Knott's test; and percent patencies determined from these measurements.

Table III. Summary of granulomatous inflammatory responses measured at necropsy in homologously infected offspring of B. pahangi-infected and uninfected female jirds.

				Granulomatous Lesions				
				Lymphatic**			Pulmonary++	
Age	Exp	Mother	n	Spermatic Cord	Total Lymph	Thrombi:Worm	DEA	BpAg
2*	1	U	6+	4.2 ± 10.2	4.2 ± 10.2	1.3 ± 3.8	8164 ± 1630	28,215 ± 4582
		I	7	7.0 ± 11.5	7.0 ± 11.5	1.5 ± 4.2	5833 ± 40	24,631 ± 6089
2	2	U	6	5.3 ± 8.6	5.3 ± 8.6	1.3 ± 1.3	6540 ± 2010	23,161 ± 9500
		I	4	5.8 ± 4.6	5.8 ± 4.6	1.5 ± 1.5	6328 ± 2328	19,058 ± 11,176
4	1	U	5	8.8 ± 7.2	9.3 ± 8.1	0.8 ± 0.6	7310 ± 879	21,537 ± 4435
		I	6	14.3 ± 17.0	15.5 ± 19.3	1.0 ± 1.1	7356 ± 1862	22,240 ± 3961
4	2	U	6	17.5 ± 13.8	19.0 ± 15.8	2.6 ± 3.0	6620 ± 1399	17,441 ± 3876
		I	7	11.6 ± 11.6	13.1 ± 11.8	1.2 ± 1.6	7795 ± 1324	15,711 ± 6279
4	3	U	6	10.7 ± 8.4	15.7 ± 16.1	2.7 ± 1.8	8120 ± 1842	20,679 ± 6069
		I	6	16.0 ± 9.3	16.8 ± 9.7	2.1 ± 4.0	6356 ± 676	21,212 ± 12,844

Footnotes for Table III.

* Age of offspring in weeks at the time of challenge infection.

+ Unequal n resulted from the deaths of jirds after infection.

** Arithmetic $\overline{X} \pm$ sd for spermatic cord duct and total lymphatic lesions; or geometric $\overline{X} \pm$ sd for the ratio of intralymphatic thrombi per intralymphatic worm.

++ $\overline{X} \pm$ SD pulmonary granulomatous areas measured around CNBS beads coated with either DEA or BpAg in um^2.

Table IV. Numbers of splenic IgG plaque-forming cells and corresponding serum IgG antibody titers to B. pahangi antigens in infected and uninfected progeny of Group I and Group U mothers.

Mother	Progeny	n	IgG PFC*	ELISA Ab+
Uninfected	Uninfected	2	0.4 ± 0.1	0.0
	Infected	3	8.0 ± 3.5	26.3 ± 13.2
Infected	Uninfected	2	0.4 ± 0.1	0.1 ± 0.1
	Infected	3	0.3 ± 0.2**	4.5 ± 2.9**

* $\bar{X}$ ± sd number of IgG plaque-forming cells to BpAg per 3.0×10^5 splenocytes determined at 5 wk postinfection.

+ $\bar{X}$ ± sd serum IgG antibody titer to BpAg as measured in ELISA units judged from a standard curve generated with known positive pooled sera.

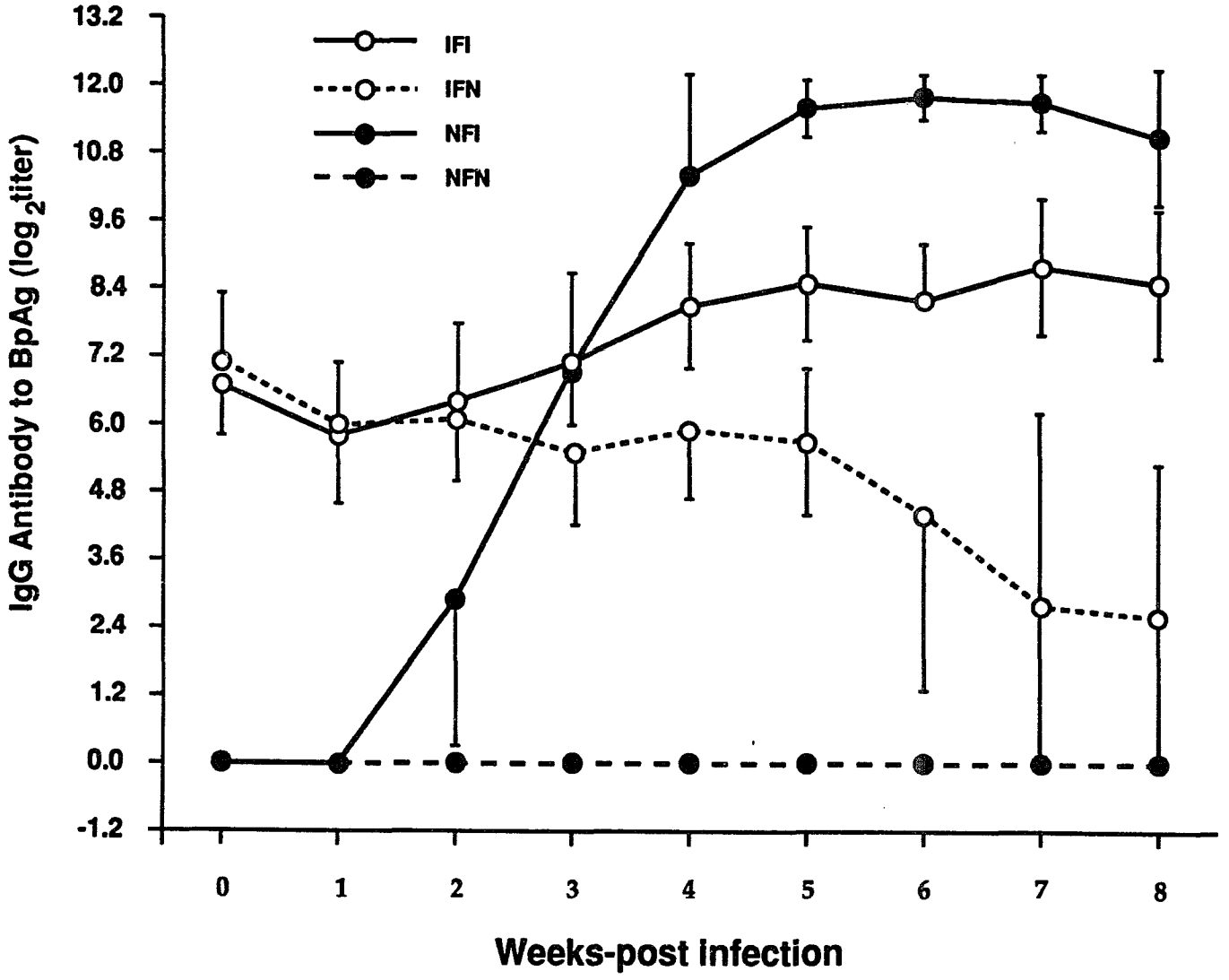
** Significantly lower ($p \leq 0.05$) than infected offspring from uninfected mothers as compared by the Student's t-test.

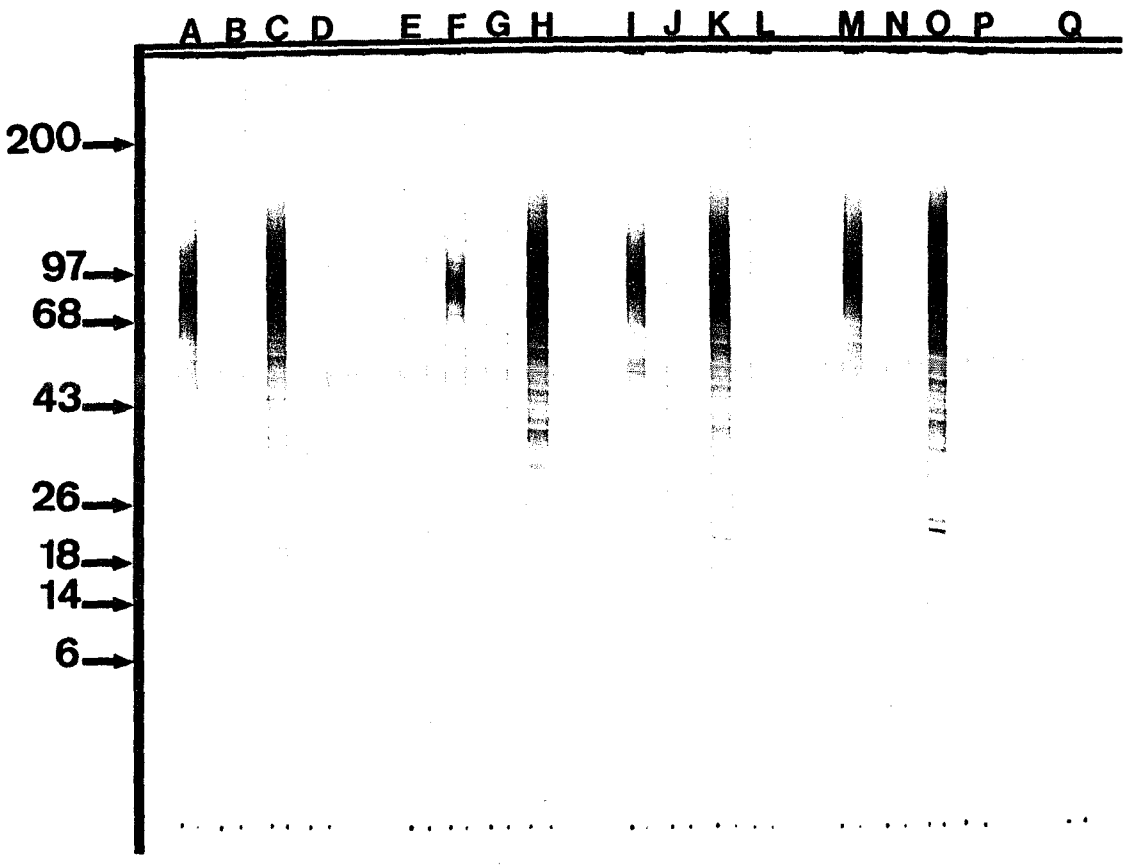
Figure 1. ELISA antibody titers to adult Brugia pahangi antigens measured in individual sera from infected male progeny from infected females jirds (o———o), infected male progeny from uninfected female jirds (●———●), uninfected male progeny from infected female jirds (o-----o), and uninfected male progeny from uninfected female jirds (●-----●). Error bars indicate the sd of the mean.

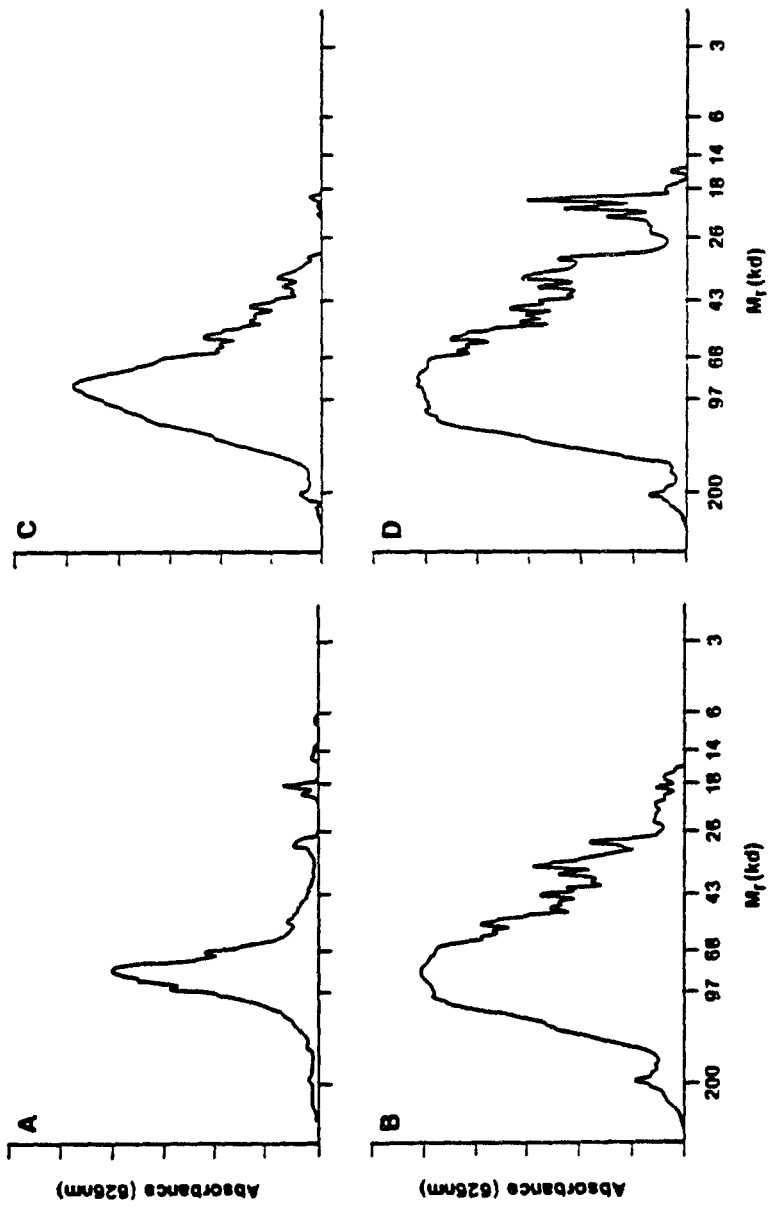
Figure 2. Western immunoblot antibody reactivity to adult Brugia pahangi antigens in pooled sera from infected and uninfected jirds from B. pahangi-infected or uninfected mothers. Lanes A, I, and M are the reactions of 8 wk postinfection pooled sera from male progeny from infected female jirds infected at 4 wk of age. Lanes C, K, and P are the reactions of 8 wk postinfection pooled sera from male progeny of uninfected female jirds infected at 4 wk of age. Lanes B and D, J and L, and N and P are the reactions of pooled sera from uninfected age-matched male progeny from infected and uninfected female jirds, respectively, for each experiment. Lanes F and H are the 8 wk postinfection reactions of pooled sera from male progeny of infected and unifected female jirds, respectively, which were infected at 2 wk of age. Lanes E and G are are the reactions of pooled sera from age-matched uninfected male progeny of infected and uninfected female jirds, respecttively, for infections

initiated in 2-wk old progeny. Lane Q represents a negative control reaction.

Figure 3. Profiles of densitometer readings from Western immunoblot strips reacted with sera from infected progeny of B. pahangi-infected or uninfected mothers against adult B. pahangi antigens. Graphs A and C are densitometer readings from Western immunoblot strips reacted with 8 wk postinfection pooled sera from male progeny infected at 2 or 4 wk of age, respectively, from infected female jirds. Graphs B and D are densitometer readings from Western immunoblot strips reacted with 8 wk postinfection pooled sera from male progeny infected at 2 or 4 wk of age, respectively, from uninfected female jirds.


IFI
IFN
NFI
NFN
IgG Antibody to BpAg (log2titer)
13.2
12.0
10.8
9.6
8.4
7.2
6.0
4.8
3.6
2.4
1.2
0.0
-1.2
0
1
2
3
4
5
6
7
8
Weeks-post infection


A B C D E F G H I J K L M N O P Q
200
97
68
43
26
18
14
6


A
B
C
D
Absorbance (625nm)
Absorbance (625nm)
200
97
68
43
25
18
14
6
3
M_r(kd)
M_r(kd)

APPENDIX A

Brugia pahangi: Depressed Cellular Immune and Granulomatous Inflammatory Responses to Adult Parasite Antigens in Immunized Progeny of Homologously Infected Female Jirds

Stephen C. Bosshardt, Sharon U. Coleman, and Thomas R. Klei

ABSTRACT - The potential for altered antifilarial immune responses to parasite antigens in offspring exposed to maternal B. pahangi infections was investigated by immunization of progeny from infected and uninfected female jirds with homologous adult worm antigens. Progeny of infected mothers immunized IP with 75ug of soluble B. pahangi antigens displayed reduced splenocyte blastogenic responses to the homologous antigens compared to similarly immunized progeny of uninfected female jirds at 10 days postimmunization. Smaller granulomatous inflammatory response areas were measured around B. pahangi antigen-coated beads embolized in the lungs of immunized progeny of infected versus uninfected females at 4 wk postimmunization. In separate experiments, pulmonary granuloma areas around antigen-coupled beads were equivalent at 10 days postimmunization in immunized groups of progeny from both maternal sources. Reductions in antigen-stimulated splenocyte blastogenesis at 10 days postimmunization, and pulmonary inflammatory responses at 4 wk postimmunization, in the progeny of infected mothers were not statistically different from immunized progeny of uninfected mothers. Immunized offspring from both maternal sources did not produce detectable amounts of serum IgG antibodies to B. pahangi antigens. These results suggest that exposure to maternal filarial infections may cause reduced antigen-stimulated in vitro lymphoproliferative and in vivo

pulmonary inflammatory responses in their progeny compared to the progeny of uninfected mothers following primary immunization with the homologous parasite antigens. Additionally, the amount of time necessary to establish suppression of these cellular responses following immunization of progeny exposed to maternal filariasis may differ.

ABBREVIATIONS: *Brugia pahangi* infective larvae [L_3], Horseradish peroxidase [HRP], 0.01M Phosphate buffered saline [PBS], 0.05% Tween 20 in PBS [PBST], Enzyme-linked immunosorbent assay [ELISA]

The expression of clinical disease in human lymphatic filariasis varies widely between infected individuals. Most patients fit into one of five categories of disease states (Otteson, 1980, 1984), but others overlap into more than one category. The reasons for the wide variety of clinical manifestations in the human lymphatic filariases is not known. Patients within a given category, however, often exhibit similarities in their immune responsiveness to parasite antigens leading to the belief that immune responses to the parasite play an important role in determining the severity of clinical disease (Otteson, 1980). An unanswered question in human lymphatic filariasis concerns the apparent immunologic and pathologic hyperresponsiveness to filarial infections exhibited by previously unexposed migrants who travel into areas endemic for filariasis (Wartman, 1947). Transmigrants into filariasis endemic areas often display an accelerated course of disease symptoms compared to the indigenous population (Partono et al., 1978; Partono, 1987). One hypothesis put forth to explain the variabilities observed in both clinical disease and immune responses to filariae states that <u>in utero</u> and neonatal exposure to filariae, filarial antigens, or maternal antifilarial immune responses can alter the responses of the progeny to subsequent homologous infections (Otteson, 1980; 1984; Piessens et al., 1987). The purpose of this investigation was to compare the immune responses

and inflammatory reactivity of progeny from Brugia pahangi-infected and uninfected female jirds to adult worm antigens following homologous primary immunization with these antigens.

Monogamous breeding pairs with age-matched females (Meriones unguiculatus) were established utilizing adult jirds obtained commercially (Tumblebrook Farms, West Brookfield, MA). Jirds were maintained on lab chow and water ad lib.

Infective L_3 of B. pahangi were obtained from crushed Aedes aegypti mosquitos counted, and inoculated as described in Chapter 1 of this dissertation. Female jirds serving as infected mother were inoculated IP with 200 B. pahangi L_3. Offspring were obtained from age-matched infected and uninfected female jirds after B. pahangi infections had developed for ≥ 100 days.

Immunization of offspring was accomplished at 4 wk of age with soluble adult B. pahangi antigens prepared as previously described (Klei et al., 1982). Jirds were administered 75ug of antigen IP in PBS in a total volume of 0.15 ml based on initial studies which had shown this level of antigen to be optimal for producing cellular immune and granulomatous responses to antigens in offspring from uninfected mothers. Unimmunized jirds received the same volume of PBS. The same antigenic preparation was used in subsequent measurements of in vitro lymphocyte

proliferation, in vivo granulomatous inflammatory responses, and serum antibody levels of immunized or unimmunized progeny from both maternal sources as described in Chapters 1 and 3 of this dissertation.

Two experimental designs were used to compare the cellular responses of age-matched progeny from infected and uninfected female jirds to immunization with B. pahangi antigens. One experiment was conducted with 6 immunized and 6 unimmunized offspring from each maternal source to measure in vitro lymphocyte blast transformation responses and in vivo pulmonary granulomatous inflammatory responses to B. pahangi antigens 10 days after primary immunization. Two experiments were each performed with 3 immunized and 2 unimmunized offspring from each maternal source to measure serum antibody responses of immunized jirds to the homologous antigen for 4 wk postimmunization. Pulmonary granulomas around B. pahangi antigen-coupled beads were also measured at the end of this 4 week period.

Splenocytes from immunized offspring of B. pahangi-infected females demonstrated markedly reduced in vitro proliferative responses to all levels of homologous antigens tested when compared to similarly immunized progeny of uninfected females at 10 days postimmunization (Fig. I). These differences, however, were statistically insignificant due to the variant response of 1 of 3 jirds in each of the immunized groups of offspring. Spleen cells from

unimmunized progeny of both maternal sources showed no responsiveness to *in vitro* stimulation with *B. pahangi* antigens. Lymph node cell blastogenic responses to antigen were much lower than those of spleen cells, but similar within groups of immunized or unimmunized progeny from both maternal sources. Lymph node cell stimulation indices from both groups of immunized progeny were approximately twice the stimulation index of 1 displayed by lymph node cells from both groups of unimmunized progeny at all levels of antigen tested *in vitro*.

Serum antibody responses to IP immunization with *B. pahangi* antigens were not detectable up to 4 wk later in offspring from either maternal source. Positive control sera used to generate the standard curve in each plate generated high OD_{630} values.

Granulomatous reactivity around *B. pahangi* antigen-coated beads embolized in the pulmonary vasculature was similar at 10 days postimmunization at approximately 17,000 um^2 in immunized progeny from both maternal sources. Granuloma areas around antigen-coated beads in the lungs of both groups of unimmunized offspring at this time ranged from 4200-4850 um^2. Granuloma areas around diethanolamine-coupled beads in the lungs of immunized or unimmunized progeny from both types of mothers at 10 days postimmunization displayed ranges of 4100-5300 um^2 and 3700-4700 um^2, respectively. Areas of pulmonary granulomatous

responses around antigen-coated beads at 4 wk postimmunization, however, were consistently smaller in immunized progeny of infected mothers compared to the immunized progeny of uninfected mothers. In two separate experiments, granuloma areas around antigen-coated beads measured 8520 ± 554um^2 versus 10,243 ± 3047um^2 and 9025 ± 4356um^2 versus 12,792 ± 2272um^2 in immunized progeny from infected versus uninfected mothers, respectively. These differences were not statistically significant.

Transplacental transmission of microfilariae has been shown to lead to a depression in both antigen- and mitogen-induced *in vitro* stimulation of splenocytes derived from the progeny of *Acanthocheilonema viteae*-implanted female Fischer rats compared to splenocytes of progeny from uninfected mothers (Haque and Capron, 1982). The suppression of splenocyte blastogenic responses to antigens at 10 days postimmunization, and pulmonary inflammatory reactivity to antigens at 4 wk postimmunization, in immunized progeny of *B. pahangi*-infected female jirds compared to the immunized progeny of uninfected mothers in this study are not thought to be due to transplacental transfer of intact microfilariae as this phenomenon has not been confirmed in jirds. Furthermore, a *B. pahangi* antigen of 105-110 kd molecular weight containing a phosphorylcholine epitope was not detectable in neonatal circulation at a time when it was detectable in the mother (Weil et al., 1990). The

possibility of maternal transmission of other filarial antigens which may lead to depressed *in vitro* splenocyte blastogenic responses to *B. pahangi* antigens, however, cannot be ruled out at this time. Interestingly, immunized jirds from infected mothers showed consistently, albeit insignificantly, smaller granuloma areas around *B. pahangi*-antigen coated beads compared to similarly immunized progeny of uninfected mothers at 4 wk postimmunization, but not at 10 days postimmunization. Experimental evidence from studies in mice suggests that maternal antibodies transfered to the progeny either *in utero* or neonatally may select against the expression of antigen-specific T cells (Martinez et al., 1986). Data taken from all three experiments suggest that the putative induction of regulatory networks in cellular granulomatous inflammatory responses to parasite antigens in the lungs of progeny of infected females requires longer than 10 days to become established, although this was not critically tested.

Immunization with *B. pahangi* antigen levels sufficient to stimulate strong pulmonary inflammatory responses to the antigen in the progeny of uninfected females were unable to stimulate measurable serum antibody responses in these offspring. Preliminary studies with jirds from uninfected mothers conducted to characterize different protocols of jird immunization with *B. pahangi* antigens indicated that immunizations with as little as 25ug of soluble antigen

resulted in low, but measurable levels of serum antibodies to the antigen 10 days postimmunization (Bosshardt and Klei, unpublished data). The reasons for the lack of serum antibody responses to B. pahangi antigens following homologous immunizations of offspring from both maternal sources with 75ug of antigen in this study are, therefore, unclear.

These data on the cellular responsiveness of offspring to B. pahangi antigens following antigen immunization suggest that maternal filarial infections may affect the expression of homologous primary immune and granulomatous inflammatory responses in their progeny. The potential effects of gestational and neonatal exposure to maternal B. pahangi infections on the homologous responses of their progeny may also be more clearly demonstrable following primary immunization compared to challenge infection due to the variability associated with immune responses and pathologic changes measured after challenge infections. Future studies into the effects of exposure to maternal B. pahangi infections on the homologous immune and pathological responses of their offspring in this model would benefit from the use of larger groups of progeny in an attempt to overcome the inherent variability of responses to this parasite.

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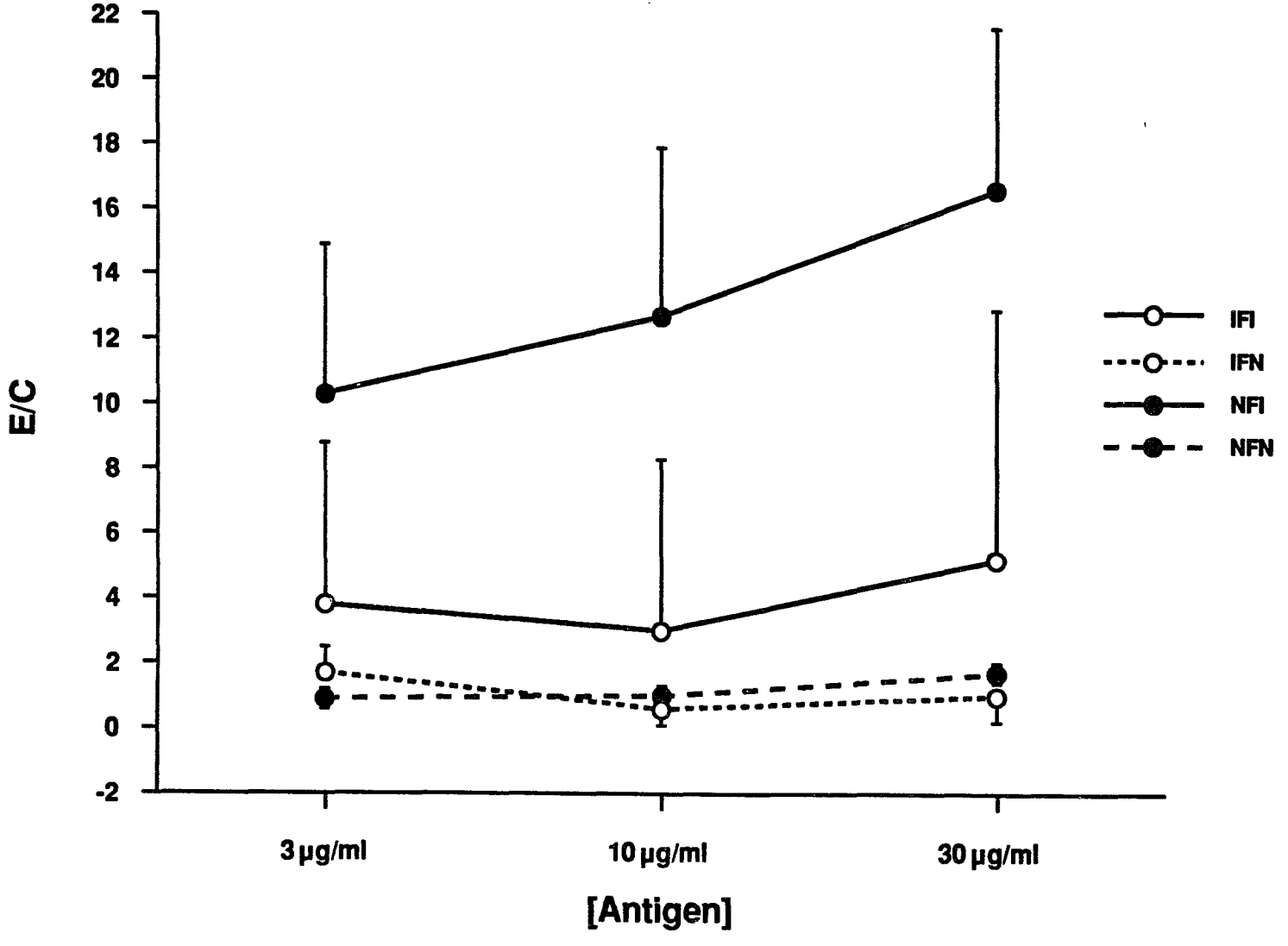
Figure 1. B. pahangi antigen-stimulated blastogenesis responses of splenocytes 10 days postimmunization with 75ug soluble B. pahangi antigens in 4-wk old progeny from infected (o———o) and uninfected female jirds (●———●), or unimmunized progeny from infected (o-----o) and uninfected female jirds (●-----●).


22
20
18
16
14
12
10
8
6
4
2
0
-2
E/C
3 µg/ml
10 µg/ml
30 µg/ml
[Antigen]
IFI
IFN
NFI
NFN

APPENDIX B

Brugia pahangi: Microfilaremias in Female Jirds (*Meriones unguiculatus*) Infected Via the Intraperitoneal Route

Stephen C. Bosshardt, Sharon U. Coleman, and Thomas R. Klei

ABSTRACT - Breeding female jirds infected with 100 B. pahangi L_3 via the intraperitoneal route were studied to determine percent patency and levels of microfilaremias. Levels of microfilariae in the circulation of IP-infected breeding female jirds reached a peak ($\overline{X}$ ± sd = 26.2 ± 62.9 mf/ 20ul whole blood) at approximately 273 days postinfection. The highest microfilaremia measured in an individual infected female was 214 mf/20ul of whole blood at 273 days postinfection. Initial percent patencies of female jirds harboring IP infections measured at 90 or 105 days postinfection in 2 groups of females was 35% and 45%, respectively. Peak patency was achieved at 158 days (55%) and 209 day (50%) postinfection in the 2 groups of infected females. Microfilariae were detectable in 75% (30/40) of IP-infected female jirds at some time during the study period. Microfilariae were detected in 1.0ml of blood by Knott's test in 72% of one group of 20 IP-infected female jirds at necropsy 404 days postinfection. Adult worm recoveries from the 2 groups of IP-infected females were 8.3 ± 12.0 and 7.1 ± 13.0 at 340 and 404 days postinfection, respectively. These data suggest that microfilaremias expressed by IP-infected female jirds display stability and longevity which may be useful for laboratory maintenance of the life cycle of B. pahangi.

The Mongolian gerbil, or jird (Meriones unguiculatus), has proven to be a permissive experimental host for the filarial nematode Brugia pahangi (Ash and Riley, 1970). Patent infections with B. pahangi are more frequently obtained in male jirds than in female jirds when infective larvae (L_3) are inoculated SC (Ash, 1971; 1973). Reasons for the increased production of patent infections of B. pahangi in male jirds compared to female jirds is not fully understood. The presence of patent infections in male jirds, however, has been attributed to the localization of adult worms within the testes and associated lymphatic vessels in SC inoculated male jirds (Ash and Riley, 1970; Ash, 1971). Both sexes of jirds can be infected via the IP route (McCall et al.,1973), but no data are available concerning the resulting microfilaremias in jirds harboring IP B. pahangi infections. The purpose of this study was to measure the levels of microfilaremias of female jirds that had been inoculated IP with B. pahangi L_3.

Adult female jirds were obtained commercially (Tumblebrook Farms, West Brookfield, MA), and housed with male jirds as monogamous pairs for breeding purposes. Jirds were fed lab chow and given fresh water ad lib.

Methods used for the collection, counting, and inoculation of B. pahangi L_3 harvested from Aedes aegypti mosquitos are described in previous chapters of this dissertation. Female jirds of approximately 15 wk of age

received 100 B. pahangi L_3 each. Levels of microfilariae in peripheral blood were determined at approximately 100 days postinfection, and every 30-45 days thereafter, by microscopic examination of 20ul of heparinized blood drawn via the retro-orbital plexus. Levels of microfilariae at necropsy were determined by the Knott's test (Knott, 1939) using 1.0ml of whole blood drawn via the retro-orbital plexus in 1 of 2 groups of B. pahangi-infected female jirds. Mean ± sd microfilaremias are expressed as numbers of microfilariae per 20ul of blood.

Mean numbers of microfilariae in 2 groups of female jirds (n=20/group) infected IP at different times with different batches of L_3 measured initially at 90 or 105 days postinfection were 1.7 ± 2.7 and 0.7 ± 1.2/20ul of blood, respectively. Peak $\overline{X}$ levels of circulating microfilariae in infected females were achieved at either 300 (26.2 ± 62.9) or 273 days postinfection (14.1 ± 48.6) in the two groups of females. The two female jirds expressing the highest microfilaremias of 214 mf/20ul and 212 mf/20ul did so at 273 and 300 days postinfection, respectively. Mean numbers of microfilariae in the blood over the 11 or 14 months of the study were 13.3 ± 8.3 and 8.3 ± 5.2 mf/20ul of blood.

Percent patency of female jirds inoculated IP with 100 B. pahangi L_3 was 35% and 45% at 90 and 105 days postinfection, respectively. Maximum percent patency was reached at 158 days (55%) and 209 days (50%) postinfection

in the two groups of IP-infected female jirds. Microfilariae were detectable in a total of 30/40 (75%) of infected female jirds at some time during the course of the study. Microfilariae were still detectable at necropsy in 20ul of whole blood in 35% of one group of females infected for 340 days, and in 72% of of the second group by Knott's test with 1.0ml of blood in females infected for 404 days.

Mean ± sd adult worm recoveries from the 2 groups of IP-infected female jirds were 8.3 ± 12.0 and 7.1 ± 13.0 at 340 and 404 days postinfection, respectively. The overall range of adult worm recoveries from individual female jirds was 0-57. Microfilariae were found in the blood or in the spleen of those female jirds (n=5) with no detectable adult worms at necropsy. Adult B. pahangi were typically found loose in the peritoneal cavity, but also in the heart and lungs, and occasionally in the uterine/ovarian lymphatic vessels. A total of 29% (11/38) of IP-infected female jirds harbored intralymphatic worms suggesting that larvae inoculated IP can migrate to intralymphatic locations. Microfilaremias were expressed in 45% (5/11) of female jirds in which lymphatic-dwelling adult worms were found indicating either, 1) that microfilariae escaped from the peritoneal cavity into circulation, or 2) the remaining 6/11 infected female jirds had intralymphatic worms that were not detected at necropsy.

Previous studies using the SC route of inoculation of B. pahangi L_3 have indicated that female jirds are less susceptible than males to infection (Ash and Riley, 1970; Ash, 1971; 1973). Female jirds infected SC also exhibited both fewer patent infections and more variable microfilaremias than similarly infected male jirds (Ash and Riley, 1970; Ash, 1971). Data reported in these previous studies in which B. pahangi infections were initiated SC in male and female jirds have indicated that approximately 85% of males became patent, while ≤ 50% of female jirds ever became patent. Female jirds infected IP in this study also demonstrated a peak mean patency of approximately 50% at times postinfection comparable to those previously reported (Ash, 1971; 1973). Differences in this study and the previous studies of Ash (1971; 1973), however, are that by approximately 105 days postinfection the patency of SC-infected female jirds had declined to only 14%. In the current study, percent patency was still 40-50% at 129 days postinfection in IP-infected female jirds. Furthermore, female jirds demonstrated a 43% patency of IP infections in infections of 340 days duration. The previous study of Ash (1971) admittedly studied fewer SC-infected female jirds than this investigation, but the data presented here indirectly suggest that IP-infected female jirds exhibit peripheral microfilaremias for longer periods of time than SC-infected female jirds.

The current study was not undertaken to directly compare the microfilaremias of male or female jirds infected either by the SC or IP routes. Data from this study, however, indicate that IP inoculation of B. pahangi L_3 into female jirds results in 40-50% patent infections, some of which express detectable levels of microfilaremias for approximately one year. Female jirds infected via the intraperitoneal route may be suitable as donors of microfilariae for the laboratory maintenance of the life cycle of B. pahangi.

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SUMMARY

The first chapter describes the presence of high titers of serum IgG antibodies to Brugia pahangi antigens in the progeny of infected female jirds. Experiments were conducted to determine the effects of this anti-B. pahangi antibody on cellular immune and pathological inflammatory responses to the homologous antigens in the lungs of uninfected progeny of both infected and uninfected mothers.

IgG antibody levels to B. pahangi antigens measured by ELISA in the sera of offspring from infected female jirds were low at birth, increased rapidly to peak titers equivalent to those of their respective mother at 2 weeks of age, and waned throughout the remaining nursing period. Rocket immunoelectrophoresis of these sera into rabbit antijird IgG indicated that levels of serum IgG in the progeny of infected and uninfected female jirds also increased to near maternal levels by approximately 2 weeks after birth. Western immunoblot analysis of infected maternal and respective neonatal serum antibody reactivity with B. pahangi antigens revealed identical patterns of antigen recognition.

The source of these neonatal antibodies to B. pahangi in the progeny of infected mothers was investigated using an in vitro antibody production assay and foster nursing experiments. In vitro antigen-stimulated antibody production assays using spleen cells from 2-week old

neonates from infected and uninfected mothers indicated that splenocytes from neither offspring group produced detectable amounts of supernatant IgG antibodies to B. pahangi antigens, despite high titers of serum IgG antibodies to these antigens in the neonates from infected mothers. Positive control cultures containing spleen cells from adult female jirds harboring intraperitoneal B. pahangi infections of 30-45 days duration produced easily measurable amounts of supernatant IgG antibodies to antigens. Progeny of uninfected mothers nursing from infected mothers demonstrated high titers of serum IgG antibodies to parasite antigens that reacted with the same parasite antigens as the foster mother in Western immunoblots. Progeny of infected female jirds nursing from uninfected mothers displayed no serum IgG antibody to B. pahangi antigens.

The effects of these neonatal serum antibodies to B. pahangi antigens on cellular immune and granulomatous inflammatory responses to the homologous antigens were measured by comparing uninfected progeny from infected and uninfected female jirds in in vitro blastogenesis assays and pulmonary lung granuloma assays. No differences in splenocyte proliferative responses between uninfected progeny of infected or uninfected mothers were measured against phytohemagglutinin, pokeweed mitogen, or B. pahangi antigens. Cellular granulomatous inflammatory responses produced around antigen-coated beads embolized in the lungs

were also of similar magnitude in progeny from both maternal sources.

The results of Chapter 1 indicated that the progeny of B. pahangi-infected female jirds had high levels of serum IgG antibodies to homologous parasite antigens which were derived from the mother through nursing. Neither the presence of these maternal antibodies in the circulation of their uninfected progeny, nor in utero and neonatal exposure to maternal B. pahangi infections, had measurable effects on the in vitro blastogenic or in vivo pulmonary granulomatous inflammatory responses to parasite antigens compared to the uninfected progeny of uninfected female jirds.

The second chapter addresses differences in age-related susceptibility of male jirds to infection with B. pahangi, and the subsequent development of lymphatic lesions and antibody responses in jirds from uninfected mothers. Data from infections in these jirds were necessary for subsequent studies of infections in the progeny of infected and uninfected female jirds.

Jirds infected at 2, 6, 10, or 15 weeks of age displayed no differences in adult worm burdens or lymphatic lesion formation associated with the presence of intralymphatic worms following challenge infections. Jirds infected at 2 weeks of age, however, had significantly fewer intralymphatic worms at necropsy, and significantly lower

titers of IgG antibodies to B. pahangi antigens at 4 and 6 weeks postinfection, than all other age groups.

The results presented in Chapter 2 suggested that the development of B. pahangi and lymphatic lesions associated with intralymphatic adult worms were not affected the age of the host when infected. Furthermore, reductions in antibody responsive observed in the youngest age group did not affect adult worm burdens or lymphatic lesion formation in these jirds. The location of adult worms in the heart, lungs, and peritoneal cavity of jirds infected at 2 weeks of age was attributed mainly to the immaturity of the usual developmental location of B. pahangi in the testes and associated lymphatics in jirds of this age.

The third chapter consists of experiments designed to test the hypothesis that in utero and neonatal exposure to maternal B. pahangi infections can alter the responses of the progeny to subsequent homologous challenge infections. These experiments were performed in an attempt to confirm earlier reports of reduced lymphatic lesion severity in the progeny of infected mothers in this model, and to add important data concerning the immune responsiveness of progeny from infected female jirds to subsequent infections.

Five experiments were performed in which jirds of either 2 or 4 weeks of age from infected and uninfected mothers were infected with B. pahangi. Results of necropsies performed at a time of maximum intralymphatic

lesion formation demonstrated no differences in either numbers of adult worms recovered, microfilaremias, or numbers of intralymphatic lesions observed, between infected offspring of either age group from infected or uninfected mothers. The formation of granulomatous inflammatory lesions around B. pahangi antigen-coated beads embolized in the lungs was also similar between age-matched infected or uninfected progeny from both maternal sources. Granulomas formed around antigen-coated beads in the lungs of progeny infected at 2 weeks of age from infected females were smaller, although insignificantly, than similarly induced granulomas in the infected progeny of uninfected females.

Antibody responses to B. pahangi antigens following infection measured by ELISA were significantly reduced in progeny infected at 2 or 4 weeks of age from infected females compared to the infected progeny of uninfected females at 4-8 weeks postinfection. The reduced antibody responses observed in the infected progeny of infected mothers were also measurable by significantly fewer splenic plaque-forming cells to B. pahangi antigens than in infected progeny from uninfected mothers at 5 weeks postinfection. Western immunoblot analysis indicated both qualitative and quantitative reductions in the reactivity of serum IgG antibodies to B. pahangi antigens in infected offspring from infected females compared to age-matched infected control

progeny at a time of maximum antibody responsiveness in both offspring groups.

The data presented in Chapter 3 suggest that reduced antibody responses in the infected progeny of B. pahangi-infected female jirds may result from a partial immune tolerance to filarial antigens induced by exposure to maternal filarial infections. The reduced antibody responsiveness observed in the infected progeny of infected mothers had no measurable effect on adult worm burdens, microfilaremias, the development of intralymphatic lesions, or antigen-specific granulomatous inflammatory responses in the lungs compared to the infected progeny of uninfected mothers.

The results of Chapters 1 and 3 indicate that the potential exists for the alteration of neonatal immune responses, possibly through the passive transfer of maternal anti-B. pahangi IgG antibodies. Studies in Appendix A measuring cellular immune and granulomatous inflammatory responses of progeny from infected and uninfected mothers following primary immunization suggest that immunization may be a more fruitful method than challenge infection in elucidating the potential influences on neonatal immunity by infected mothers in the jird-B. pahangi model.

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M.S. THESIS:

Antifecundity Effects of Chronically Infected Baboon (Papio cynocephalus) Serum on Schistosoma mansoni In vitro.

Major Professor: Dr. Raymond T. Damian

PhD DISSERTATION:

Brugia pahangi: Effects of maternal filariasis on the responses of their progeny to homologous infection.

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ACADEMIC AND PROFESSIONAL APPOINTMENTS

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Research Technician II, University of Georgia Department of Poultry Parasitology; 1980-1981

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Graduate Research Assistant, University of Georgia Department of Zoology; 1982-1984

Research Technician II, University of Georgia Department of Zoology; 1984-1985

Graduate Research Assistant, Louisiana State University Department of Veterinary Microbiology and Parasitology; 1985-present

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Current research interests include the immune responses and associated pathologic responses of chronic human parasitic diseases. Studies have concentrated on

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RESEARCH GRANTS AND CONTRACTS:

STUDENT CO-INVESTIGATOR:

Effects of *in utero* exposure to *Brugia* on subsequent development of homologous infections, immune responses and associated lesions. World Health Organization(to T.R. Klei-PI) $55,000.00. 1987-1989.

HONORS, AWARDS, AND FELLOWSHIPS:

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PUBLICATIONS:

Bosshardt, S.C., and Damian, R.T. 1986. Serum factors from infected baboons inhibit oviposition and cause unpairing of *Schistosoma mansoni in vitro*. Journal of Parasitology **72**: 583-587.

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Bosshardt, S.C., McVay, C.S., Coleman, S.U., and Klei, T.R. Brugia pahangi: Circulating antibodies to adult worm antigens in uninfected progeny of homologously infected female jirds. (submitted)

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DOCTORAL EXAMINATION AND DISSERTATION REPORT

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Major Field: Veterinary Medical Sciences/Parasitology

Title of Dissertation: Brugia pahangi: Effects of maternal filariasis on the responses of their progeny to homologous infection.

Approved:

Major Professor and Chairman

Dean of the Graduate School

EXAMINING COMMITTEE:

Date of Examination:

9/28/90